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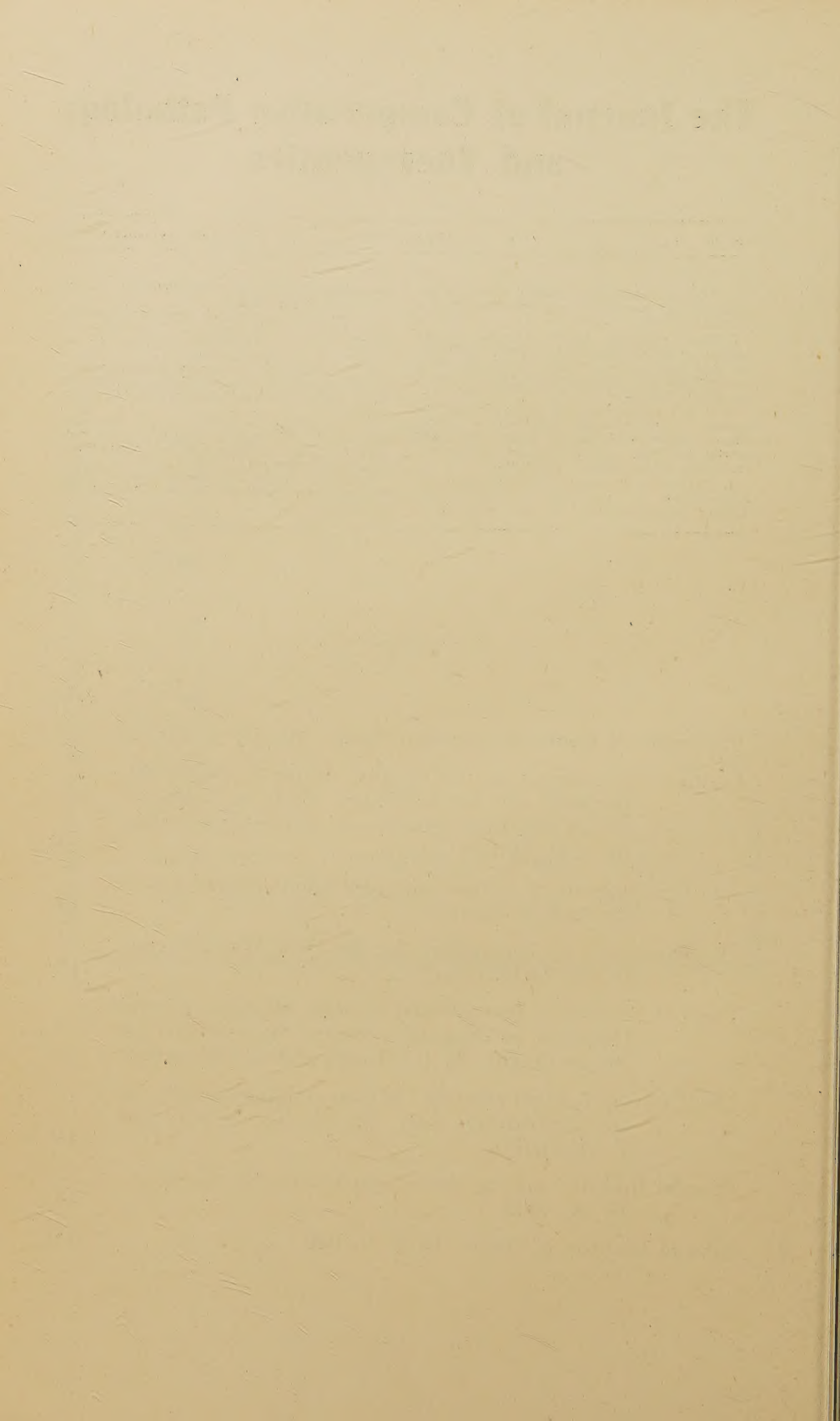
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PLASMODIA OF COMMON EGYPTIAN BIRDS *

By

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INTRODUCTION

MALARIAL infections in birds have aroused great interest in recent years. The causal parasites are assumed to undergo exactly the same stages of development as those of human malaria. Avian malaria has thus come to be regarded as having certain real advantages in the laboratory study of malaria. Where wild birds in a locality are found to be infested, a large amount of experimental material becomes available, for among them the incidence of infection is usually high and in non-fatal cases the course of the infection can be followed for years or even for the whole life of the bird. Avian parasites have recently been propagated in laboratories in temperate countries on a large scale where their chief interest has been the study of certain phases of malaria infection which had remained obscure and also to test the activity of the newer synthetic, antimalarial compounds such as mepacrine, pamaquine and paludrine which possess certain well-defined pharmacological characteristics that were not to be found in the time-honoured remedy, quinine. Study in birds has revealed hitherto unknown stages, namely, the exoerythrocytic stages, in the plasmodial life-cycle. In the light of the discovery of these stages in which the organisms assume the role of tissue parasites, the so-called cryptozoites, malariologists have attempted to explain by analogy the frequency of relapses in human malaria. Hitherto these exoerythrocytic stages have not been detected with certainty by visual examination in the human subject, though evidence for their occurrence has become very strong from the results of chemotherapeutic research. Although a large variety of birds abounds in Egypt, they have not been investigated for malarial infection and references to the subject as it affects Egypt are almost non-existent. The author, therefore, decided that a preliminary investigation into the subject was desirable.

MATERIAL AND METHODS

Specimens, mainly blood films, were supplied from the different poultry breeding stations of the Ministry of Agriculture at Inshas,

* Summary of a thesis presented for the Degree of M.V.Sc., Fouad I University, Cairo, carried out at Fouad I Institute and Tropical Diseases Hospital, Cairo, under the supervision of Prof. A. Halawani, May, 1946.

Fayoum, Sakha, Gimmesa, Dokki and Sidce, from the birds at the Egyptian Zoological Gardens, and from the Egyptian Royal Hunting Club at Cairo as well as from various other localities. Passerine birds were trapped from various areas for examination and inoculation experiments. A list of the different species examined is given in Table I. Attempts were made to rear most of them under strict hygienic conditions. Blood taken from either the leg or comb was exclusively used. Giemsa, Leishman and May-Grünwald stains were tried, but the one which gave most satisfactory results and was adopted as a routine was Giemsa. At autopsy, tissues from various internal organs were fixed in Zenker's fluid

TABLE I
EGYPTIAN SPECIES OF BIRDS EXAMINED FOR MALARIAL INFECTION AND
THEIR LOCAL NAMES

Number of birds examined	Species of birds	Local name
65	<i>Passer domesticus niloticus</i>	House sparrow
7	<i>Fringilla c. coelebs</i>	Chaffinch
4	<i>Oenanthe l. lugens</i>	Morning chat
2	<i>Oenanthe hispanica melanoleuca</i>	Black-eared wheatear
4	<i>Oenanthe o. oenanthe</i>	Wheatear
8	<i>Carduelis c. niediecki</i>	Golden finch
5	<i>Merops orientalis cleopatra</i>	Little green bee-eater
3	<i>Merops superciliosus persicus</i>	Blue-cheeked bee-eater
3	<i>Merops apiaster</i>	European bee-eater
2	<i>Pycnonotus barbatus</i>	White-vented bulbul
4	<i>Coccothraustes c. coccothraustes</i>	Hawfinch
150	<i>Columba livia domestica</i> and <i>Streptopelia senegalensis aegyptiaca</i> }	{ Domestic pigeon Palm dove
500	<i>Gallus gallus domesticus</i>	Domestic fowl
120	<i>Anas rubripes tristis</i>	Black duck
120	<i>Chenalepex aegyptiacus</i>	Goose
120	<i>Meleagris gallopavo</i>	Turkey, domestic
105	<i>Athene noctauglaux</i> and <i>Falco tinnunculus rupicolaeformis</i> }	{ Owl Egyptian kestrel
52	<i>Milvus migrans aegyptius</i>	Black kite
3	<i>Galerida cristata migricans</i>	Crested lark
2	<i>Passer domesticus chephreni</i> Phillips	House sparrow

for 24 hours. For sections, Hewitt's (1939) device for staining was adopted nearly throughout the whole work because it gave the most satisfactory results in differentiating between the parasites and the host cells. In experimental transmission, the method adopted was the inoculation of healthy birds into the breast muscle with 0.5 c.c. of infected blood diluted 1 : 10 in isotonic citrate-saline. The incubation period stated in each case was the number of days which elapsed before the first appearance of parasites in the blood. Meanwhile careful precautions were observed to exclude accidental infection from mosquitoes, etc.

DESCRIPTION AND MEASUREMENTS OF PLASMODIA INVESTIGATED IN THE
BLOOD AND INTERNAL ORGANS

Out of a total of 62 naturally infected Egyptian birds, 40 per cent. were found to harbour species of *Plasmodia* and 60 per cent. species of *Haemoproteus*. The species of *Plasmodia* were: *P. praecox*, *P. cathemerium*, *P. elongatum*, *P. rouxi*, *P. nucleophilum* and a single specimen of *P. gallinaceum* from a fowl of an European breed.

Plasmodium praecox (Plate I) Danilewski, Grassi and Feletti, (1890-91).

The different stages observed in the blood of birds were nearly similar to those described by previous authors. The following points

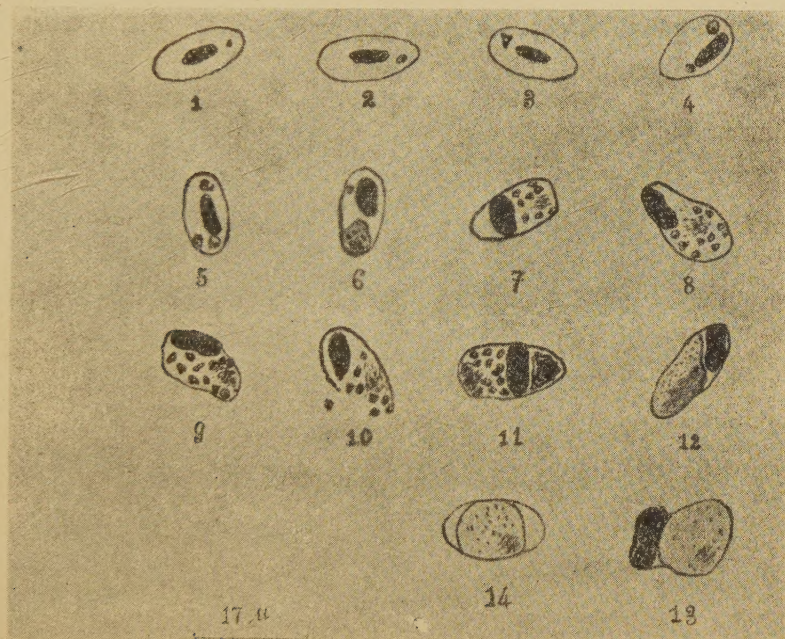


PLATE I.—Diagrammatic representation of the various forms observed in the successive stages in the life cycle of *Plasmodium praecox*.

of detail deserve mention. In the trophozoites (Plate I, 1-5) (Fig. 1) more than one nucleus was sometimes seen especially in the irregular and ring-shaped forms. No pigment granules were noted in this stage. The parasitised red cells were unaltered and their nuclei not displaced except in cases of multiple infection with more than one trophozoite (Plate I, 4-5) (Fig. 2) or with a trophozoite together with another stage (Plate I, 6). The trophozoites varied from 1.7 to 3.4 μ in diameter. Manwell's observation (1935) of the occurrence of these forms mostly at the poles of the cell nucleus was confirmed.

Small schizonts developed into mature spheroidal forms producing from eight to 12 merozoites (Plate I, 6-11) (Fig. 3); an irregular mass of brown pigment granules was observed at one side. Some parasitised red cells were so packed with merozoites that their shape was completely altered and their nuclei greatly displaced; free nuclei found outside their red cells resulted from massive infections. Schizonts varied from 6.8 to $10.2\ \mu$ in diameter. Each merozoite was $1.7\ \mu$ in diameter. Occasionally, the nucleus of a parasitised red cell was displaced to the transverse position (Plate 1, 11).

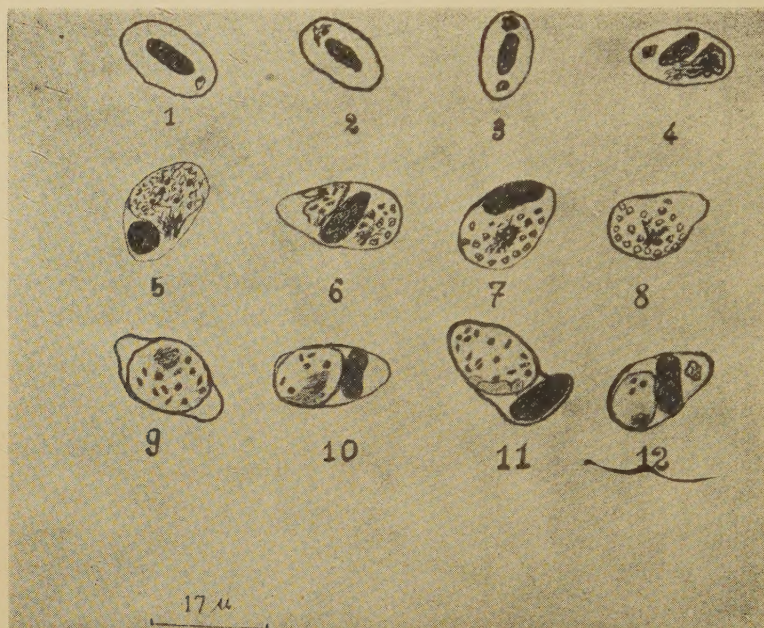


PLATE II.—Diagrammatic representation of the various forms observed in the successive stages in the life cycle of *Plasmodium cathemerium*.

Round or spheroidal gametocytes (Plate I, 12-14) (Fig. 4) occurred alongside the nucleus of infected red cells. This observation is in contrast to that of Hartman (1927), who described this stage as crescentic in shape in European species of birds. Fine, dust-like, dark brown pigment granules were distributed all over their cytoplasm especially in the macro-gametocytes. The gametocytes varied from 6.8 to $10.2\ \mu$ in diameter and in some cases they filled the red cell so completely as to displace the nucleus to the extreme edge or push it completely outside. Occasionally, more than one gametocyte was observed to infect one red cell or a gametocyte together with another stage.

P. praecox was observed in sparrows and a plasmodium indistinguishable from it was found in pigeons. It was most common, however, in the former birds, rising to as high as 80 per cent. among passers. The incubation period was seven to eight days.

Plasmodium cathemerium (Plate II) Grassi and Feletti (1890-91), Hartman (1927). This species exhibited morphological characteristics similar to those of *P. praecox* excepting that it was larger in size. It is usually considered very hard to differentiate *P. praecox* from *P. cathemerium*, except from the nature of the pigment. The present author would therefore wish to stress the following points



PLATE III.—Diagrammatic representation of the various forms observed in the successive stages in the life cycle *Plasmodium elongatum*.

of difference. Schizonts (Plate II, 4-8) reached 11.9μ in diameter and produced more than 20 merozoites. Gametocytes (Plate II, 9-11) (Fig. 5) were large, more than filling the normal interior of the infected red cells, which then became completely altered in appearance and showed displacement of their nuclei. In several instances gametocytes were observed in the plasma outside the ruptured infected red cells. They varied between 10.2 and 11.9μ in diameter and contained oval or round, coarser, dark brown pigment granules, which were numerous in the macro-gametocytes. The incubation period was four to five days.

Plasmodium elongatum (Plate III) Grassi and Feletti (1890-91), Boyer (1924), Hartman (1927) and Huff (1930). This has been com-

monly known as the "elongated parasite." It is a true plasmodium (*i.e.*, it is not a *Haemoproteus*) in that it can be transmitted from one host to another by blood inoculation as was confirmed in the present work. Its trophozoite stages (Plate III, 1-4) were found to resemble those of *P. praecox*, the elongated form being seen at one side of the nucleus towards the apex. No pigment granules were noticed in the elongated form except a single granule, or sometimes two granules clumped together. This form tapered at one end and caused no alteration or displacement of the cell nucleus except in cases where more than one parasite was present. Trophozoites varied between 1.7 and $3.4\ \mu$ in diameter but the elongated ones reached $5.1\ \mu$ in length. Schizonts (Figs. 6 and 7) were found near one apex of the red cell nucleus, which was then usually crowded into the other end of the cell where it lay transversely (Plate III, 12). Ten to 20 merozoites were noticed in mature schizonts together with a mass of clumped pigment granules at one side. Mature schizonts were rarely observed in the peripheral blood. In some cases schizonts together with other stages were found to infect one red cell (Plate III, 12) displacing its nucleus to the transverse position. Schizonts varied from 5.1 to $10.2\ \mu$ in diameter. Gametocytes were elongated (Fig. 7) and lay alongside the nucleus, with smooth margins and round ends and extended from one apex to the other of the red cell. The displacement of the nucleus was complete and not, as previously reported by Huff, only slight. The red cell was usually increased in size along its longitudinal axis. Pigment granules were large, round, evenly grouped and placed rather towards one side. The gametocytes measured from 3.4 to $5.1\ \mu \times 8.5$ to $10.2\ \mu$. In some cases more than one gametocyte together with other stages was noticed to infect one red cell (Plate III, 9-13). Sparrows, especially the passers, were most frequently infected with *P. elongatum*. In several instances this species was found in association with other plasmodia, particularly *P. cathemerium*, as a mixed infection in the same host. The incubation period was eight to ten days.

Plasmodium rouxi (Plate IV) Sergeant, Ed. and Et., and Catanei (1928). This was found on rare occasions in Egyptian sparrows especially the passers. Its trophozoites were similar to those of *P. praecox*, but a fourth type was observed which was merely the ring shaped form with a cytoplasmic tail-like structure having at its end a dot-like red chromatin nucleus. On this account this form of the parasite resembled an insect-net (Plate IV, 3); it was $3.4\ \mu$ in length. More than one form of trophozoite has been recorded as infecting one red cell. Neither pigment granules nor displacement of the nucleus was observed. The small quadrangular schizonts varied between 4.2 and $8.5\ \mu$ and were placed near the poles causing neither alteration in the parasitised red cell nor displacement of its nucleus. This characteristic shape of the schizonts is due to the fact that each schizont produces four merozoites only (Fig. 8), each occupying one corner of the schizont (as in Plate IV, 6-7). The

residual mass of dark refractile pigment granules was usually situated near the centre of the quadrangular schizonts. Each one of the merozoites which thus did not exceed four in number appeared conical in shape and about $1.2\ \mu$ in length. In a very few instances free merozoites were seen displacing the nucleus of the infected red cell (Plate IV. 9). Schizonts always assumed a polar position. The gametocytes, which were very rarely encountered in the peripheral circulation, were usually very small and elongated. Two only were found in a blood film. They were from 3.4 to $4.2\ \mu \times 6.8$ to $8.5\ \mu$ in size and caused no displacement of the cell nucleus. The macrogametocyte was of a deeper hue and contained more

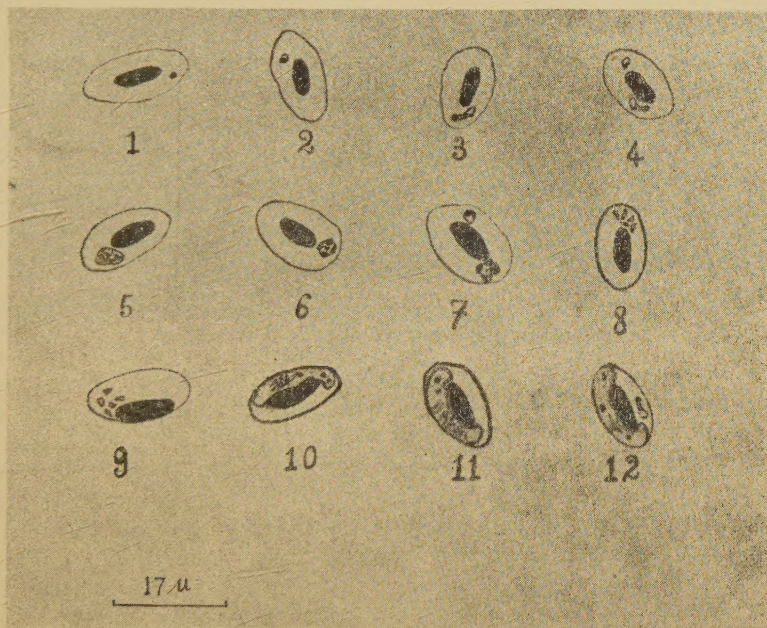


PLATE IV.—Diagrammatic representation of the various forms observed in the successive stages in the life cycle of *Plasmodium rouxi*.

pigment granules which were refractile, unequal in size and collected two or three at one position. This type was found mostly in sparrows especially the passers. The incubation period was ten days.

Plasmodium nucleophilum (Plate V), Manwell (1935). This rare species, recently discovered, was found infecting the house sparrow. As the name implies, the mature forms of the successive stages were usually seen closely applied to the nucleus of the infected host red cells (Plate V, 6-11). It is a somewhat small parasite, its stages resembling similar stages of *P. rouxi* rather closely and it also forms elongated gametocytes. For these reasons it was at first

mistaken for *P. rouxi*. Later, however, it became evident that there were sufficiently well-marked and constant morphological characteristics to make it necessary to place this parasite in a new species. Schizonts (Fig. 10) were found to be small, measuring from 4.8 to 8.9μ . They were nearly always found at the poles in contact with the nucleus which was not even slightly displaced until the moment of liberation of the pear-shaped merozoites which varied in number from five to seven. In red cells in which there is multiple infection the nucleus is often displaced. Gametocytes (Fig. 11) were very rare in the peripheral blood. They were elongated, alveolar and closely applied to the cell nucleus which was not displaced. They varied from 3.4 to $4.2 \mu \times 6.8$ to 8.5μ and contained two to three pigment granules, usually collected together. The gametocytes found in some cases were seen accompanying other stages in one cell.

Plasmodium gallinaceum (Plate VI), Brumpt (1935). The author encountered this parasite once only in a dead fowl of European breed. None was found in indigenous fowls in spite of careful search, and their susceptibility awaits further investigation.

Table II depicts the characteristics of the various stages of the foregoing species.

EXOERYTHROCYTIC STAGES OF PLASMODIA FOUND TO OCCUR IN THE TISSUES OF THE INTERNAL ORGANS

In addition to the plasmodial stages occurring in the blood cells, the present writer also observed other stages infecting the cells of the internal organs of killed or naturally dead birds. These forms were seen in cases infected with *P. praecox*, *P. cathe-merium* and *P. elongatum*. Unpigmented forms, blue in colour, were demonstrated in the reticulo-endothelial system of these birds. In most of the cases in the present work in which this phenomenon was observed, the parasites showed a tendency to localise more especially in the brain, spleen, liver and lungs and, rarely, in the kidneys and heart muscles. In the brain, the cells principally affected were the capillary endothelial cells together with the monocytes and macrophages in which all stages from small trophozoites to schizonts were observed. The parasites were also observed to infect some of the nerve cells (Fig. 12), A and B. In the other organs also, the parasites appeared to infect cells of the lymphoid-macrophage, or reticulo-endothelial, system, comprising monocytes, macrophages, endothelial cells, Küpffer cells and small and large lymphocytes.

OTHER HAEMOSPORIDIA OBSERVED IN THE BLOOD OF EGYPTIAN BIRDS

Elongated sausage shaped cytoplasmic bodies were found in the red cells of sparrows, pigeons and black ducks. They were usually situated on one side of the nuclei, causing partial or complete displacement of the nucleus contrary to statements made by other



PLATE V.—Diagrammatic representation of the various forms observed in the successive stages in the life cycle of *Plasmodium nucleophilum*.



PLATE VI.—Diagrammatic representation of the various forms observed in the successive stages in the life cycle of *Plasmodium gallinaceum*.

TABLE II
DESCRIPTION AND MEASUREMENTS OF THE DIFFERENT STAGES OF AVIAN PLASMODIA INVESTIGATED

Plasmodia	Egyptian hosts	Trophozoites	Schizonts	Gametocytes	Percentage of infection	Incubation period	Remarks
<i>P. praecox</i> ..	<i>Passer d. niloticus</i> <i>Merops o. cleopatra</i> <i>Fringilla c. coelebs</i> <i>Oenanthe l. lugens</i> <i>Coccothraustes c.</i> <i>Passer d. Alex.</i> <i>Oenanthe o. oenanthe</i> <i>Columba l. domestica</i>	Round, irregular and ring forms 1.7 to 3.4 μ . No pigment granules, causing displacement to the red cell nucleus	Spheroidal or round, 6.8 to 10.2 μ , producing 8-12 merozoites, causing partial or complete alteration of the red cell and nucleus. Pigment granules were dark brown and dust-like diffusely distributed		70	7-8 days	Plate I
<i>Plasmodium</i> sp. similar in morphology to <i>P. praecox</i>							
<i>P. cathemerium</i>	<i>Passer d. niloticus</i> <i>Coccothraustes c. co.</i> <i>Passer d. Alex.</i>	As <i>P. praecox</i>	As <i>P. praecox</i> , but larger, 11-9 μ , producing more than 20 merozoites	As <i>P. praecox</i> . 10.2-11.9 μ . Pigment granules were coarse, round, oval or rod shaped	20	4-5 days	Plate II
<i>P. elongatum</i> ..	<i>Passer d. niloticus</i> <i>Coccothraustes c. co.</i>	Round, irregular, ring, and elongate. The last reaches 3.4 μ in length. No pigment granules, and no displacement to red cell nucleus	Small, quadrangular, 4.2 to 8.5 μ . Polar, producing 10 to 12 merozoites with clumped pigment granules	Elongated, 3.4 to 5.1 $\times$ 8.5 to 10.3 μ . In diameter with displacement to red cell nucleus; pigment granules are large, round and grouped especially at the poles	20	8-10 days	Plate III
<i>P. rouxi</i> ..	<i>Passer d. niloticus</i>	As <i>P. praecox</i> , together with the insect-net shaped form. 3.4 μ in length. No pigment granules, no displacement to red cell nucleus	Quadrangular, small, 4.2 to 8.5 μ , producing four merozoites only as well as clumped mass of pigment granules	Small, elongated, 3.4 to 4.2 $\times$ 6.8 to 8.5 μ in diameter. Displacement to red cell nucleus was rare, contained two to three pigment granules	10	10 days	Plate IV
<i>P. nucleophilum</i>	<i>Passer d. niloticus</i>	As in <i>P. rouxi</i>	Small, quadrangular or spheroidal, 4.8 to 8.5 μ , in close contact with the red cell nucleus at the poles which was not displaced except in condition when were liberated merozoites; produced from five to seven merozoites. Pigment granules were clumped at one side	Like those of <i>P. rouxi</i> , but closely applied to red cell nucleus which was not displaced	10		Plate V
<i>P. gallinaceum</i>	<i>Gallus g. domesticus</i>	As in <i>P. praecox</i>	As in <i>P. praecox</i> but bigger, producing about 25-30 merozoites	As in <i>P. praecox</i> but bigger and rare in peripheral circulation			Plate VI From a European fowl

authors. Sometimes they surrounded both ends of the nucleus of the parasitised red cell and, in some cases, were found to fill up all the cytoplasm. In blood films no stages were observed except these gametocytes and those merozoites which were destined to form new gametocytes. The gametocytes therefore belonged to the genus *haemoproteus*. They were sky blue in colour and each contained an irregular chromatin mass together with dust-like yellowish brown pigment granules grouped in very small circles more especially at the poles of the parasite. The macrogametocytes appeared deeper in colour and some alveolar. More than one stage was sometimes observed to infect one red cell. The mature gametocytes measured $3.4 \times 11.9 \mu$ in diameter. The *Haemoproteus* parasites observed in different species of birds were essentially similar but those infecting sparrows appeared to be more slender than those infecting pigeons and ducks. A larger type investigated by the writer in the blood of the black duck was observed to stain more deeply than is usual for the gametocytes of other members of the genus, confirming the original observation of Herman (1938). This parasite was provided with large yellowish brown pigment granules present in varying size and differing in number from eight to 12, and occurring at one pole or both poles of the haemosporozoan.

The different species observed in Egyptian birds may therefore be listed as follows:

<i>Haemoproteus</i> sp. in <i>passers</i>	}	In sparrows
.. .. <i>merops</i>		
.. .. <i>carduelis</i>		
.. .. <i>fringella</i>		
.. .. <i>coccothraustes</i>		
<i>Haemoproteus columbae</i>		In pigeons
<i>Haemoproteus hermani</i> (*) n. sp.		In black ducks

* A species indistinguishable from the above has been described by Herman (1938) as a new species in the black duck of Europe, but it was not named by him.

BRIEF ACCOUNT OF THE MACROSCOPIC AND MICROSCOPIC CHANGES OCCURRING IN THE INTERNAL ORGANS AND BLOOD OF INFECTED CASES

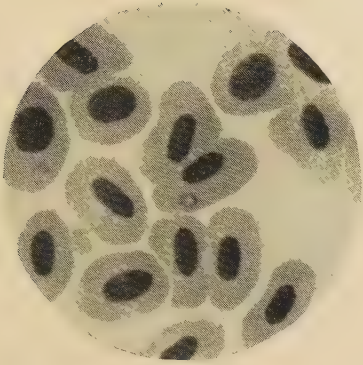
A further investigation of the gross changes in the different body tissues has been undertaken. Some of these, such as those occurring in the spleen and bone marrow are in harmony with the findings of MacCallum and Shulamite Ben-Harel (1923) in cases of *haemoproteus*-infected and *plasmodium*-infected birds. The changes in size and colour of the bone marrow due to atrophy of its fat particles as well as to the hyperplastic condition in the blood elements, was first observed by Bizzozzero and Torre in anaemic birds.

The following detailed pathological account gives a description of the changes in the liver, heart, kidneys, brain and blood. On

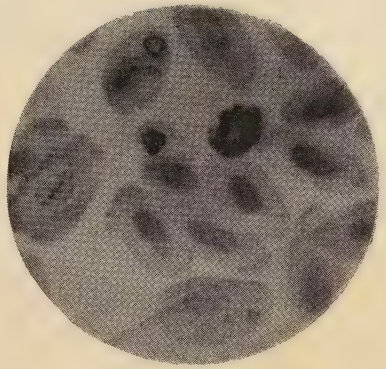
dissection much subcutaneous dropsical fluid was found and in most cases the viscera were swollen. The heart was pale, hypertrophied and flabby and its blood vessels congested. In some cases there was fatty degeneration and a large quantity of pericardial effusion. Sections revealed accumulations of pigment inside the congested capillaries, the endothelial cells of which were also seen rarely to contain fragments of parasites and to show slight degeneration, with pigment granules distributed diffusely within them. The brain was usually congested and of a slightly deeper colour than normal. In sections the capillaries were dilated and filled with parasitised or non-parasitised red cells as well as clumps of pigment. In some cases different forms of parasites were seen phagocytised by the endothelial cells lining the walls of the capillaries. These cells were often swollen and projected into the lumen of the vessel. Pigment granules were also found within the endothelial cells scattered throughout the brain tissue. The parasitised nerve cells in most instances showed karyolytic degeneration. The liver was always enlarged. Its colour varied greatly from steel grey to olive, chocolate, or black. It was mottled, due to variations in the degree of local congestion and fatty degeneration, while the cut surface showed marbling. Microscopically, the blood vessels together with the sinuses were congested and dilated and contained brown pigment and parasites free or within red cells. Küpffer cells were swollen, polymorphic and sometimes contained pigment and parasites. Pigment was usually seen scattered irregularly in the liver tissue. Slight degeneration or atrophy affected some hepatic cells. The columns of cells were seen separated by the dilated blood vessels which were blocked with large pigmented phagocytes. The kidneys were swollen, pale and reticulated, with nutmeg-like mottling. Microscopically, the glomeruli were congested and the blood vessels were dilated and filled with parasitised or non-parasitised red cells together with clumps of pigment granules. Pigment, and occasionally a few parasites, were seen phagocytised by the endothelial cells but pigmented granules were also irregularly distributed throughout the whole tissue. Blood appeared pale, thin and watery as it oozed out of blood vessels of infected birds. The number of red cells was reduced beyond the normal, *i.e.*, there appeared to be excessive destruction of these cells due to actual infection. Most of the infested red cells showed irregularities in size and shape and they were pale in colour containing less haemoglobin than the normal cells. Small red cells were commonly seen. There was actual leukopaenia. The percentage of the large mononuclear cells was increased.

CONCLUSIONS

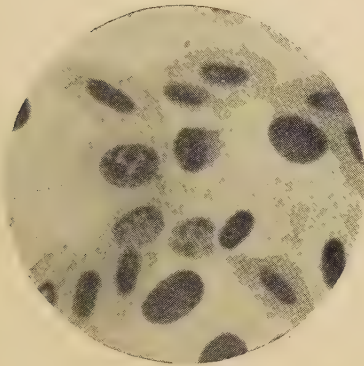
Egyptian birds (1,279) belonging to different species have been investigated for malarial infection. The morphology and life-cycle of the various species of parasites, the exoerythrocytic forms of the



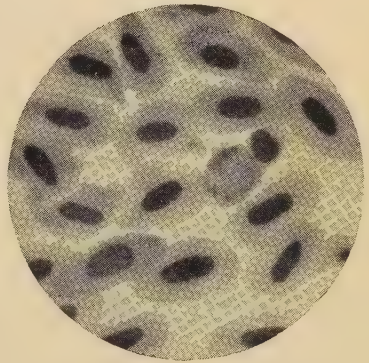
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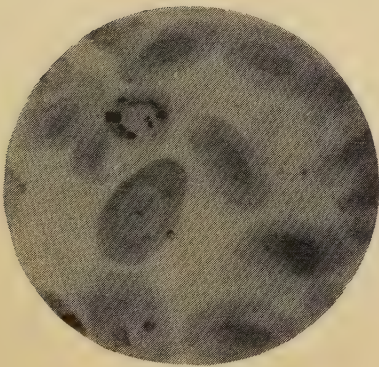
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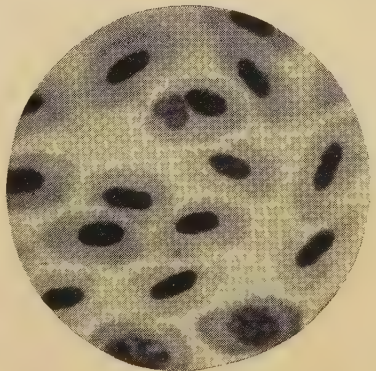
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FIG. 1.—*P. praecox*. Trophozoites stage in blood of *Merops orientalis cleopatra*.

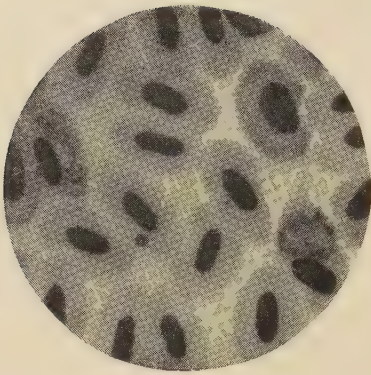
FIG. 2.—*P. praecox*. Trophozoites stage in blood of *Passer domesticus niloticus*, more than one form in one red cell displacing its nucleus.

FIG. 3.—*P. praecox*. Mature schizont in blood of *Merops orientalis cleopatra*, the schizont producing from 8 to 12 merozoites pushing the nucleus of the red cell to one side.

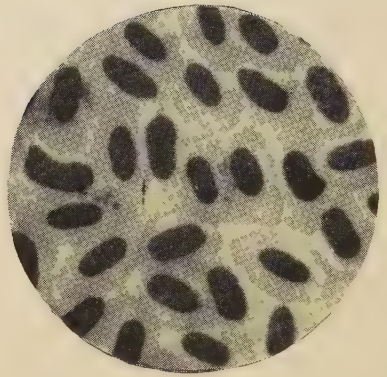
FIG. 4.—*P. praecox*. Macrogametocyte in blood of *Merops orientalis cleopatra* filling nearly all the red cell displacing its nucleus completely and provided with numerous fine dust-like pigment granules.

FIG. 5.—*P. cathemerium*. Macrogametocyte stage in blood of *Passer domesticus niloticus* provided with oval or rod-shaped coarse pigment granules.

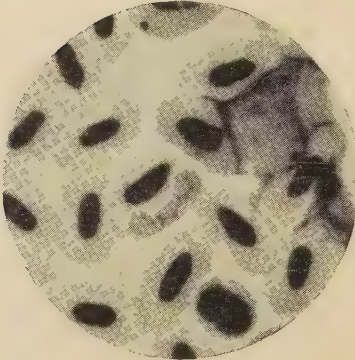
FIG. 6.—*P. elongatum*. Mature schizont in blood of *Coccothraustes c. coccothraustes*, situated at one pole of the displaced red cell nucleus producing 10 merozoites.



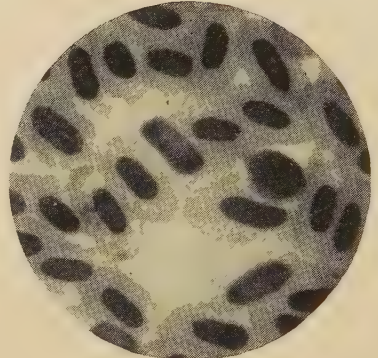
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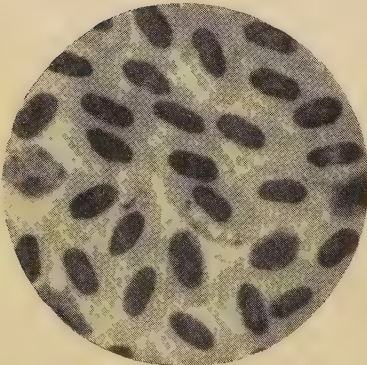
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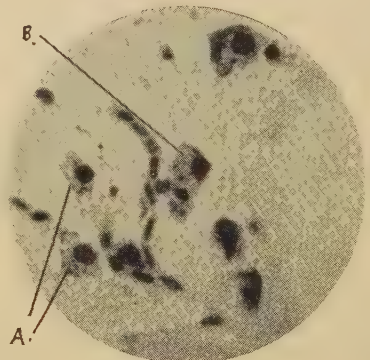
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FIG. 7.—*P. elongatum*. Gametocyte stage in blood of *Passer domesticus niloticus*, elongated alongside the displaced red cell nucleus with smooth round ends.

FIG. 8.—*P. rouxi*. Trophozoites and mature schizont in blood of *Passer domesticus niloticus*, the mature schizont produced four pear-shaped merozoites only together with the residual body.

FIG. 9.—*P. rouxi*. Gametocyte in blood of *Passer domesticus niloticus*, elongated causing no displacement to the red cell nucleus.

FIG. 10.—*P. nucleophilum*. Mature schizont in blood of *Passer domesticus niloticus*, producing six merozoites together with the residual body causing very little displacement to the red cell nucleus.

FIG. 11.—*P. nucleophilum*. Free merozoites and gametocytes in blood of *Passer domesticus niloticus*, the latter is elongated, alveolar and in-contact to the red cell nucleus which is not displaced.

FIG. 12.—*P. elongatum*. (A) Free merozoites inside a nerve cell which is altered and has nuclear displacement; (B) Ring form trophozoites in the cytoplasm of another nerve cell.

parasites together with the pathological changes in the hosts have been studied. The incubation period was determined for the most frequently occurring species by means of transmission experiments.

Nearly all the species of malarial parasites described in this work on Egyptian birds have been previously reported outside Egypt.

The author has observed a new species of *Haemoproteus* in Egyptian black ducks, morphologically identical with that described by Herman (1938) in European ducks as a new species but not named by him. The author after studying its morphological characters and confirming the finding that it is different from *H. columbae* has therefore named it *Haemoproteus hermani*, n. sp.

Plasmodium praecox was found in *Passer domesticus niloticus*, *Merops orientalis cleopatra*, *Fringilla c. coelebs*, *Oenanthe l. lugens*, and a similar species in *Columba livia domestica*.

Plasmodium cathemerium was found in *Passer domesticus niloticus* and *Coccothraustes c. coccothraustes*.

Plasmodium elongatum was found in *Passer domesticus niloticus* and *Coccothraustes c. coccothraustes*.

Plasmodium rouxi and *P. nucleophilum* were found in *Passer domesticus niloticus*.

Plasmodium gallinaceum was encountered once only in a European variety of the domestic fowl.

Members of the genus *Haemoproteus* have been met with in the following birds: *Merops orientalis cleopatra*, *Passer domesticus niloticus*, *Fringilla c. coelebs*, *Coccothraustes c. coccothraustes*, *Carduelis c. niediecki*, *Columba livia domestica* and *Anas subripes tristis*.

ACKNOWLEDGMENTS

I am greatly indebted to Professor A. Halawani, Director of Fouad I Institute for Research and Tropical Diseases Hospital, Cairo, for his supervision and guidance throughout the work. My thanks are also due to J. T. Edwards, Visiting Professor and Temporary Dean of the Faculty of Veterinary Medicine, for his interest in this work and help in preparing this paper for publication.

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FURTHER OBSERVATIONS ON THE CURATIVE VALUE OF DIMIDIUM BROMIDE (PHENANTHRIDINIUM 1553) IN *TRYPANOSOMA CONGOLENSE* INFECTIONS IN BOVINES IN UGANDA

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INTRODUCTION

THE curative value of dimidium bromide against *Trypanosoma congolense* infections in bovines in Uganda has been well established by previous workers (Carmichael and Bell, 1944; Randall and Laws, 1947). At the eleventh meeting of the Standing Veterinary Research Committee held at Kabete, Nairobi, in 1947, it was, however, decided that further experiments should be conducted at various veterinary research stations in East Africa, more especially to test the relative therapeutic effects of the two phenanthridinium compounds, Phenanthridinium S. 897 [7-amino-9(p-amino-phenyl)-10 methyl phenanthridinium chloride] and Phenanthridinium 1553 [2:7-diamino-9-phenyl-10-methyl-phenanthridinium bromide] against *T. congolense* infections in bovines.

At the Entebbe laboratory, it was found impracticable to work with Phenanthridinium S. 897, but the following experiment was carried out with Phenanthridinium 1553.

AIMS AND METHOD OF THE EXPERIMENT

The 57 cattle in this experiment were pure or mixed Zebu and Ankole indigenous breeds and had not previously been infected with trypanosomes. They were all infected on February 25th, 1947, with a *T. congolense* "Busimi" strain maintained in this laboratory in mice since isolation from a bovine on November 2nd, 1945, and subinoculated from the 91st passage. Twenty-four mice, which had received a strong infective inoculum, were used for the 57 bovines. The mice were bled on the third day after infection when trypanosomes were numerous and the blood diluted with normal saline 1 in 4, each bovine received 2 c.c. of the inoculum.

A 3 per cent. solution of dimidium bromide was used in the present experiment. Previous experience in Uganda had suggested that the local reaction and the risk of subsequent necrosis and sloughing of the skin over the injection area was greater following the subcutaneous route of injection than when the intramuscular method was adapted. In a small preliminary experiment carried out in February, 1947, the reactions to subcutaneous injections were never serious, but this route was not considered suitable for field use and was not tried in this experiment. Half the cattle under

experiment, therefore, received dimidium bromide intramuscularly (i/m) and half intravenously (i/v).

Since Randall and Laws (1947) had noted a better response to dimidium bromide, when treatment was given after the disease was well established than during the early stages, 54 of the bovines in the present experiment were divided into three groups "A," "B" and "C"—each consisting of 18 head. Group "A" was treated on the 14th day after infection, Group "B" on the 24th day, and Group "C" on the 34th day.

Randall and Beveridge (1946, 1947) have shown that doses of dimidium bromide at 1 mg./kg. were non-toxic and curative in 95 per cent. of cases, while doses in the region of 2 mg./kg. could be toxic and cause photosensitisation. In the present experiment the 18 bovines in each group were sub-divided into three sub-groups with six bovines in each, sub-group (a) being treated at 1 mg./kg., (b) at 1.5 mg./kg., and (c) at 2 mg./kg. In each sub-group three bovines received the dimidium bromide i/v and three i/m. The dosage and method of administration for each group is shown in Tables I, II and III. Group "D" consisted of three untreated control animals.

Thick blood smears were taken daily from all 57 bovines, stained in Giemsa without fixation and examined for trypanosomes. The cattle in each group were weighed weekly and examined for signs of emaciation, anaemia and photosensitisation.

RESULTS

Efficacy of the drug

Group "A."—The age, body weight, dosage of Phenanthridinium 1553, and results of treatment of the bovines in Group "A" are given in Table I.

All the cattle were infected on February 25th, 1947, and were positive to *T. congolense* seven to ten days later. Trypanosomes were easily found in all cases in thick blood smears on the day of treatment (March 11th, 1947), 14 days after infection.

In this group excellent results followed treatment. Trypanosomes were scanty in the peripheral blood of all the bovines 32 hours after treatment, but at the 48th hour only nine were positive, five in sub-group (a) and two in both sub-groups (b) and (c). At the 56th hour only four bovines in sub-group (a) showed very scanty trypanosomes, and on the 72nd hour all were negative. There was no difference in the rate of disappearance in bovines treated by the i/m and i/v routes respectively; only one animal relapsed.

Group "B."—The age, body weight, dosage of Phenanthridinium 1553, and results of treatment of the bovines in Group "B," are given in Table II.

This group was treated on March 21st, 1947, ten days later than those in Group "A." All showed trypanosomes in the peripheral blood for 14 to 17 days before treatment and *T. congolense*.

TABLE I
RESULTS OF TREATMENT OF 18 BOVINES WITH PHENANTHRIDINIUM 1553
14 DAYS AFTER INFECTION WITH *T. congolense*

Sub-group	Bovine number and sex	Age	Weight in lb.*	Rate of dosage	Dose in c.c. of phen. 3% sol.	Result on 24th week
" A "	5298 ♂	4 years	635	1 mg./kg. i/m.	9.6	Cure
	5404 ♀	2 years 10 months	448	"	6.7	"
	3870 ♀	9 years	702	"	10.6	Relapse
	5347 ♂	3 years 6 months	516	1 mg./kg. i/v.	7.8	Cure
	5488 ♀	1 year 10 months	280	"	4.2	"
	5243 ♀	4 years 6 months	476	"	7.2	"
" B "	5385 ♂	2 years	427	1.5 mg./kg. i/m.	9.6	Cure
	5481 ♀	1 year 11 months	392	"	8.8	"
	5043 ♀	5 years	552	"	9.5	"
	5350 ♀	3 years 6 months	501	1.5 mg./kg. i/v	11.3	"
	5399 ♀	3 years	376	"	8.5	"
	5279 ♀	4 years 2 months	450	"	10.2	"
" C "	5291 ♀	4 years	476	2 mg./kg. i/m.	14.4	Cure
	5521 ♀	1 year 9 months	252	"	7.6	"
	5124 ♀	5 years	450	"	13.6	"
	5368 ♂	3 years 5 months	472	2 mg./kg. i/v.	14.2	"
	5410 ♀	2 years 10 months	494	"	14.8	"
	5290 ♀	4 years	440	"	13.2	"

* Weight date of treatment

TABLE II
RESULTS OF TREATMENT OF 18 BOVINES WITH PHENANTHRIDINIUM 1553
24 DAYS AFTER INFECTION WITH *T. congolense*

Sub-group	Bovine number and sex	Age	Weight in lb.	Rate of dosage	Dose in c.c. of phen. 3% sol.	Result on 24th week
" A "	5312 ♀	3 years 9 months	528	1 mg./kg. i/m.	8	Cure
	5353 ♀	3 years 6 months	442	"	6.7	"
	4967 ♀	6 years 6 months	647	"	9.8	"
	5594 ♀	1 year 2 months	148	1 mg. kg. i/v.	2.2	"
	5595 ♀	1 year 2 months	185	"	2.8	"
	5041 ♀	5 years 2 months	680	"	10.3	"
" B "	5403 ♀	2 years 6 months	416	1.5 mg./kg. i/m.	9.5	Cure
	5409 ♀	2 years 10 months	506	"	11.5	"
	4983 ♀	6 years 3 months	601	"	13.6	"
	5218 ♀	4 years 9 months	576	1.5 mg./kg. i/v.	13.1	"
	5400 ♀	2 years 6 months	381	"	8.6	"
	5599 ♀	1 year 2 months	150	"	3.4	"
" C "	5502 ♀	1 year 10 months	300	2 mg./kg. i/m.	9.1	Cure
	5503 ♀	1 year 10 months	340	"	10.3	"
	4970 ♀	6 years 6 months	538	"	16.3	"
	5247 ♀	4 years 5 months	317	2 mg./kg. i/v.	9.6	" " P "
	5281 ♀	4 years 2 months	426	"	13.9	"
	5343 ♀	3 years 8 months	575	"	17.5	"

" P " = Photosensitisation

were easily found in all cases on the day of treatment. Results in this group were also excellent. At the 32nd hour after treatment smears from two bovines in both sub-groups (b) and (c) were negative to trypanosomes, and on the 48th hour the remaining 14 animals were also negative. There were no relapses or deaths and only one case of photosensitisation.

Group "C."—The age, body weight, dosage of Phenanthridinium 1553 and results of treatment of the bovines in Group "C," are given in Table III.

TABLE III.
RESULTS OF TREATMENT OF 18 BOVINES WITH PHENANTHRIDINIUM 1553
34 DAYS AFTER INFECTION WITH *T. congolense*

Sub-group	Bovine number and sex	Age	Weight in lb.	Rate of dosage	Dose in c.c. of phen. 3% sol.	Result on 24th week
"A"	3889 ♀	9 years	558	1 mg./kg. i/m.	8.5	Relapse
	5310 ♂	3 years 10 months	410	"	6.2	Cure
	5340 ♀	3 years 9 months	484	"	7.3	"
	5299 ♀	3 years 11 months	454	1 mg./kg. i/v.	6.9	"
	5585 ♀	1 year 3 months	168	"	1.7	"
	5601 ♀	1 year 1 month	196	"	3.0	Relapse
"B"	5355 ♀	3 years 5 months	468	1.5 mg./kg. i/m.	10.6	Cure
	5301 ♀	3 years 10 months	518	"	11.8	"
	5295 ♀	4 years	360	"	8.2	"
	5025 ♀	5 years 6 months	492	1.5 mg./kg. i/v.	11.3	"
	5235 ♀	4 years 2 months	520	"	11.8	Died (drug toxicity)
	5444 ♀	2 years 4 months	434	"	9.8	Cure
"C"	5603 ♀	1 year 1 month	124	2 mg./kg. i/m.	3.7	Cure
	5262 ♀	4 years 3 months	593	"	17.9	Died (drug toxicity)
	5242 ♀	4 years 6 months	420	"	13.0	Cure
	5221 ♀	4 years 8 months	454	2 mg./kg. i/v.	13.7	"
	5370 ♀	3 years	431	"	13.0	Destroyed
	5417 ♀	2 years 9 months	404	"	12.2	Cure

This group was infected on the same date as those in Groups "A" and "B" and was treated 34 days after infection on March 31st, 1947. Most of them, therefore, had been showing trypanosomes in their blood for 24 to 27 days previous to treatment. The immediate response to dimidium bromide treatment was again excellent. Trypanosomes were scanty in the peripheral blood after 24 hours, and on the 30th hour all those treated at 2 mg./kg. and three treated at 1.5 mg./kg. were negative. All the remaining bovines were negative on the 40th hour. Two animals, however, relapsed in sub-group (a) and one died in each sub-group (b) and (c), the deaths being due not to trypanosomiasis but to the toxicity of the drug.

Group "D" (Controls).—This group consisted of three untreated bovines, infected at the same time as the previous groups, *i.e.*, on February 25th, 1947. No. 5421, aged two years and five months, died 31 days after infection and showed the typical oedematous, anaemic *postmortem* appearance of *T. congolense* infection. The two remaining animals—No. 5597, aged ten months, and No. 5582, aged eleven months—showed a persistent blood infection for about 51 days after infection, and from then until the end of the experiment irregular relapses occurred. On the 20th week their condition was improving and they appeared to be on their way towards recovery and auto-sterilisation.

Changes in body weight

The changes in body weight are not easily interpreted (Fig. 1). One might expect a steady gain in weight after treatment, but this was not achieved by any of the sub-groups. Thus in Group "A" the initial gain was interrupted by an unexpected loss in sub-groups (b) and (c) on the eleventh week after infection and all sub-groups lost weight on the 18th to 21st weeks. In Groups "B" and "C," all three sub-groups showed the lowest averages during the 11th and 12th weeks respectively, and there was also some fluctuation on the 19th and 20th weeks. From the 20th week onwards, however, there was a gain in all sub-groups, as shown in Table IV.

TABLE IV
AVERAGE GAIN OR LOSS IN BODY WEIGHT IN ALL SUB-GROUPS ON THE
20TH AND 24TH WEEKS COMPARED WITH INITIAL WEIGHT

Groups	20th week			24th week		
	"A"	"B"	"C"	"A"	"B"	"C"
Sub-group "A"	+ 1	— 40	— 37	+ 3	— 14	— 11
" " "B"	+ 16	+ 29	— 7	+ 17	+ 48	+ 32
" " "C"	+ 31	+ 3	— 53	+ 40	+ 29	— 25
Total gain or loss in lb.	+ 48	— 8	— 97	+ 60	+ 63	— 4

Thus Group "A" had gained 12 lb. in the four weeks, Group "B" had converted a loss of 8 lb. into a gain of 63 lb., and Group "C" had recovered 93 lb., but was still 4 lb. below the initial average weighings. The greatest losses were in Group "C," sub-group (c). At the end of the 24th week, however, all the cattle appeared in good condition with the exception of the relapse cases Nos. 3870 and 3889,

Toxicity and Relapses

In Group "A" (Table I), No. 3870, aged nine years, was the only animal to relapse. This cow, which showed trypanosomes in the blood on May 7th, 57 days after treatment at 1 mg./kg. i/m, was retreated on June 5th at 2 mg./kg., and calved normally on

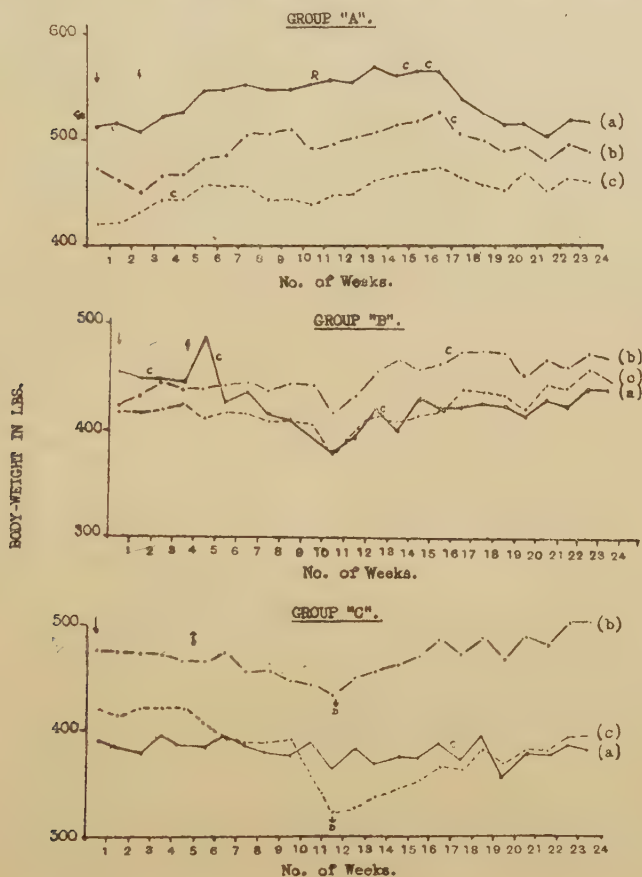


FIG. 1. Average weekly body weights of the bovines in each sub-group for 24 weeks after infection with *T. congolense*.

- C = Cow calved.
- R = Relapse.
- ↓ = Infected with *T. congolense*.
- ↑ = Treated with dimidium bromide.
- D = Death of one animal.

June 14th, the calf weighing 51 lb. The haemoglobin value was 12 grammes per 100 c.c. blood and packed cell volume 39 per cent. on the 24th week. The animal was then considered free of *T. congolense*, but on the 27th week it developed a uterine bacterial infection from which it died.

In Group "B" (Table II) there were no relapses or deaths, but No. 5247, treated at 2 mg./kg., showed signs of photosensitisation seven weeks after treatment.

The skin on the outer surface of the ears, on the dewlap, on the inner surface of the fore-arm and flexor surface of the carpal joint, the inner surface of the flank above the hock, and on the ischeal tubers became tough and scaly. These affected areas were almost entirely restricted to those parts covered by white hair; the general areas of the body and back were unaffected. The muzzle and head were also unaffected and there was no lachrymation. The skin eventually peeled off all the affected areas but the damage was only superficial; the hair follicles were unaffected and new hair coat was able to develop. There was considerable loss of weight (Fig. 2) but the condition was never serious and, as there were little signs of photophobia, the cow was not stabled; recovery was slow but uneventful. The condition of the cow on the 18th week is shown in Fig. 3. The blood picture was not studied in any great detail but there was some anaemia and icterus during the

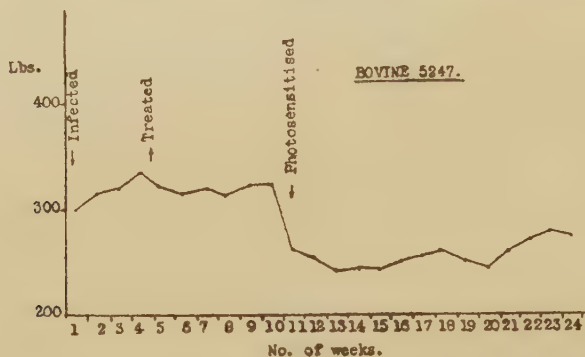


FIG. 2. Chart showing body weight of B.5247, infected with *T. congolense* and treated at 2 mg./kg. dimidium bromide 24 days later.

period when photosensitisation symptoms were most prominent. On the 14th week of infection, the red-cell count was 4.13 millions per c.c. but rose on the 18th week to 5.43 millions per c.c. and on the 23rd week to 6.66 millions per c.c. The haemoglobin was reduced in a very similar manner, being 7.1 gms. per 100 c.c. on the 14th week and 10.6 gms. on the 23rd week. There was a high resting blood sugar without glucosuria. The results of two intravenous glucose-tolerance tests showed a high type of curve with failure to return to the resting blood level after two hours and glycosuria continued throughout the period of the test. Some liver dysfunction must, therefore, have accompanied the photosensitisation and this point is being further investigated.

In Group "C" (Table III) two animals relapsed in sub-group (a) and one died in each of sub-groups (b) and (c). No. 5601 and No. 3889 relapsed on the 37th day and on the 45th day after treatment respectively. Both were retreated on June 5th at 2 mg./kg. dosage. The trypanosomes were very scanty in the blood of No. 5601, 24 hours after treatment, but were still easily found in the blood of

No. 3889; both were negative 48 hours after treatment. No. 3889, the oldest animal in Group "C," lost weight from the day of infection. The haemoglobin estimation was 5.80 grammes per 100 c.c. of blood on the 18th week and 6.67 grammes on the 24th week. No trypanosomes were found after the second treatment, but the animal continued to lose weight and died on the 27th week. The younger growing animal, No. 5601, showed no great change in weight and made a good recovery. Fig. 4 shows the weekly weights of these two animals during the period of the experiment.

No. 5262 died 43 days after treatment at 2 mg./kg. This animal had gradually lost weight from the date of infection, the weight on

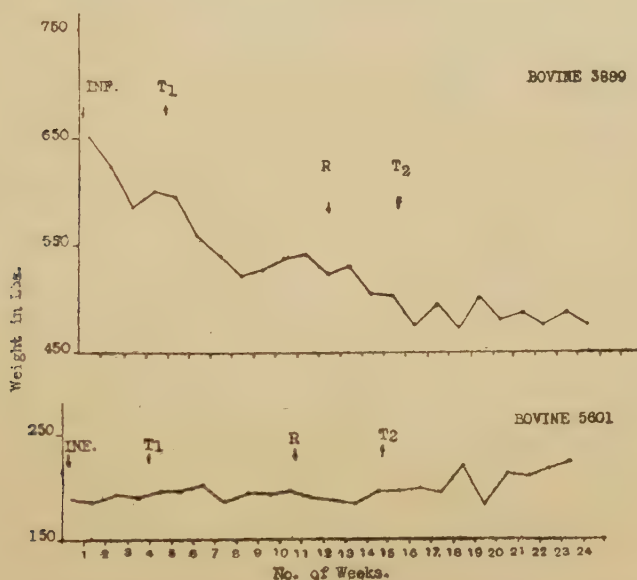


FIG. 4. Charts showing body weights of B.3889 and 5601 which relapsed after treatment of 1 mg./kg. [INF. = Original infection with *T. congolense*; T₁ = treatment at 1 mg./kg.; R = relapse, T₂ = retreatment at 2 mg./kg.].

tenth week being 82 lb. less than the initial weight. The eleventh weekly weighing, a few days before death, showed a loss of 128 lb. in seven days.

The skin, especially on the muzzle and ears, showed early signs of photosensitisation. On *post-mortem* examination most organs showed evidence of jaundice and congestion, the liver especially being enlarged, fatty and congested. There were signs of inflammation in the alimentary tract. Microscopically, the most marked changes were seen in the liver, sections from various parts showing widespread necrosis and fatty degeneration. The area of least damage in each lobule was around the central vein where the structure of the cells was maintained, but even here many of the cells showed fatty changes and there were some signs of nuclear changes with eosinophilic staining in many of the lobules. The

lining cells of the sinusoids were destroyed whilst many of the Kupffer cells were swollen and desquamated into the sinuses. There were no signs of liver-cell regeneration. In the peripheral areas of the lobule the damage was almost complete, the cells being necrosed and their cytoplasm was heavily charged with fat globules. There was some cellular infiltration in these areas and eosinophiles were numerous. Around the portal tract there were definite areas of small cell infiltration. Congestion was marked with what appeared to be small haemorrhages in some areas. The kidneys showed little signs of destruction; the glomeruli were congested but otherwise normal. There was also considerable congestion throughout the medulla and debris within the renal tubules.

No. 5235, killed *in extremis* 46 days after treatment at 1.5 mg./kg., had gradually lost weight from the fifth week after infection and showed a loss of 52 lb. on the week before death. There were signs of early photosensitisation on the muzzle. *Post-mortem* changes were very similar to those of No. 5262, but less marked. Jaundice and congestion were general. The destruction of liver cells was focal and not widespread, as in No. 5262, whilst areas of necrosis and fatty degeneration occurred around certain of the portal tracts. Eosinophiles were again prominent but the cellular infiltration around the portal tracts was less marked. In both these bovines death was attributed to delayed poisoning by dimidium bromide.

The occurrence of relapses, deaths and photosensitisation in all groups is summarised in Table V.

TABLE V
SUMMARY OF RELAPSES, DEATHS AND PHOTOSENSITISATION IN 54 CATTLE
TREATED WITH DIMIDIUM BROMIDE

<i>Bovine number</i>	<i>Group</i>	<i>Treatment</i>	<i>Result</i>	<i>Days after infection</i>	<i>Days after treatment</i>
3870	"A"	1 mg./kg.	Relapse	71	57
3889	"C"	"	"	79	45
5601	"C"	"	"	71	37
5247	"B"	2 mg./kg.	Photosensitised	73	49
5262	"C"	"	Death from drug toxicity	77	43
5235	"C"	1.5 mg./kg.	" " "	80	46

Blood examination

Haemoglobin and packed cell volumes were estimated on the 18th week and the 24th week; the haemoglobin values are shown in Fig. 5. In Group "A" only two bovines on the 18th week and three on the 24th week were below 10 g. per 100 ml. blood, so that most of the animals had approached normal values by the 18th week. In Group "B," the respective numbers were 12 and four animals, denoting a considerable recovery from the 18th to 24th weeks. In Group "C," seven animals were below 10 g. both on the 18th week and on the 24th week after infection. The

delayed treatment in Groups "B" and "C" had no doubt caused a greater anaemia and recovery was therefore slower than in Group "A." The mean corpuscular haemoglobin concentration on the 24th week in all animals, except two in Group "A," one in Group "B," and three in Group "C," was within the normal range of 30 to 35 per cent.

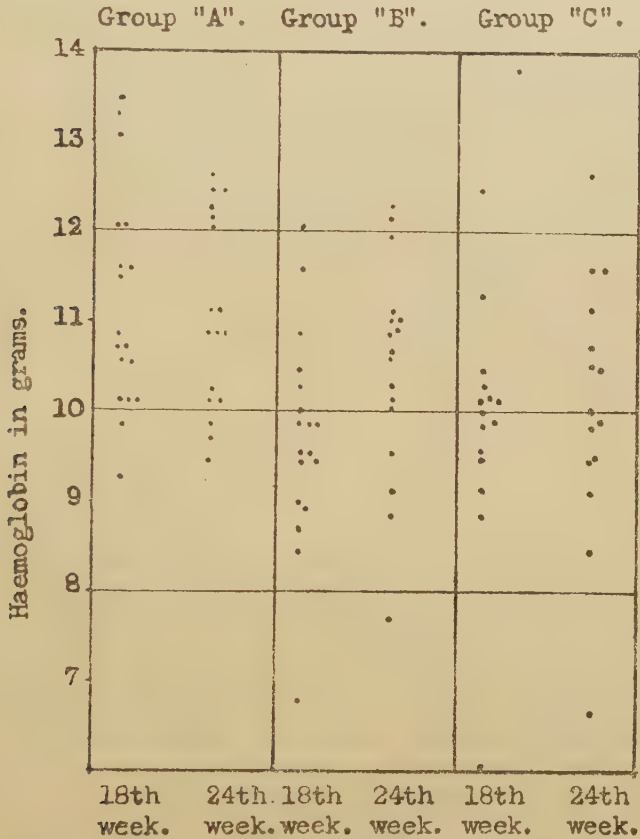


FIG. 5. Haemoglobin estimations (Sable-Hallige) on the three experimental groups of bovines during the 18th and 24th weeks after infection.

DISCUSSION

The results of this experiment confirm the efficacy of dimidium bromide in the treatment of *T. congolense* in bovines since the trypanosomes disappeared from the peripheral blood in the 54 animals within 30 to 72 hours after treatment. The rate of disappearance was hastened when the drug was given at high dosage levels and infected animals were treated when infection was well established. The three relapse cases in the group of 18 animals treated at 1 mg./kg. gives a relapse rate of 16 per cent. The two aged animals eventually

died in spite of retreatment, but the younger bovine made a good recovery.

It would appear that when infections are treated with dimidium bromide, a period of "depression" or crisis occurs from 70 to 80 days after infection, when relapses, deaths and photosensitisation are liable to occur. This period is also marked by a loss in the average body weights of many of the sub-groups. It is unfortunate for practical purposes that this depression period cannot be related to the date of treatment as the time of infection can rarely be predicted with accuracy in field cases.

In this connection it is interesting to note that French and Hornby (1935) noted a period of crisis in untreated *T. congolense* cases in adult bovines three to four months after infection, whilst Fiennes, Jones and Laws (1946) reported an increasing anaemia in untreated cases of *T. congolense* infection until the 12th week during which deaths in adult cattle are likely to occur.

Calver (1945), working with two strains of *T. congolense* in mice, noted a "remarkable regularity of relapse" after treatment with subcurative doses, and reported that treatment with different drugs over a considerable range of dosage and stages of infection did not alter this regularity. He attributes this phenomenon to an immunity-reaction which develops after non-curative doses and which is relay tively constant for each strain. In the present experiments the 1 mg./kg. dose was not sufficient to free the blood system entirely of trypanosomes in the case of Nos. 3870, 3889 and 5601, but the immunity reaction was sufficient to prevent the appearance of trypanosomes in the peripheral blood until the 71st and 79th day after the original infection, although the relapses in these cases cannot be related to the time of treatment.

The toxicity of dimidium bromide for cattle requires much further investigation since photosensitisation has been an embarrassing feature of treatment with this drug in field cases in Uganda. The irregularity with which deaths and photosensitisation occur in the field, the delay in the development of symptoms until approximately six weeks after treatment, and the fact that cases can occur in the field with doses as low as 1 mg./kg., suggest that the drug alone is not the sole cause of these specific toxic effects.

In an experiment at this laboratory early in 1947, eight animals suffering from a chronic *T. vivax*-*T. uniforme* infection were treated with a freshly prepared solution of dimidium bromide at 2 mg./kg. and only one mild case of photosensitisation developed.

In the present experiment one batch of drug was made up at a strength of 3 per cent. on March 2nd and stored in the laboratory. The solution was therefore nine days old when Group "A" was treated, 19 days old for Group "B," and 29 days old for Group "C." One case of photosensitisation developed in Group "B," and two deaths from toxicity occurred in Group "C." The toxicity of the drug on storage, therefore, requires investigation.

It is possible that the stage of *T. congolense* infection may be a contributory factor in the toxicity of dimidium bromide. If this is the case the present experiment would suggest that treatment early in the disease (Group "A") will cause little ill-effects, later treatment (Group "B") will cause typical photosensitisation to develop in a small percentage of cases, while delayed treatment (Group "C") may cause death before typical signs of photosensitisation have time to develop. This point also requires investigation.

CONCLUSIONS

Six bovines in a group of 18 infected with *T. congolense* were treated with dimidium bromide at 1 mg./kg. 14 days after infection; five were cured and one aged animal relapsed. Six, treated at 24 days, were all cured, but in six treated at 34 days only four were cured and one aged animal and one calf relapsed. At this dosage, therefore, the relapse rate, which was highest amongst aged animals, was 16 per cent. of animals treated; 15 animals (84 per cent.) were cured.

With a dose of 1.5 mg./kg., two groups of six animals were cured when treated at 14 days and 24 days respectively; five were cured and one died of dimidium bromide poisoning when treated at 34 days. Seventeen animals (94 per cent.) were cured at this dosage rate.

With a dose of 2 mg./kg., six animals treated at 14 days were all cured, five were cured and one animal was photosensitised when treated at 24 days and five were cured and one died of dimidium bromide poisoning when treated at 34 days. Sixteen animals (89 per cent.) were cured at this rate of dosage.

Delay in treating infected animals until 24 days after infection gave the best results, but further delay (34 days) caused an increased relapse rate at 1 mg./kg. dosage and two deaths from poisoning at higher dosage levels. Delayed treatment also caused slower recovery from the anaemia due to the *T. congolense* infection, and greater loss of body weight. Delayed treatment had the slight advantage of causing the trypanosomes to disappear more quickly from the blood after treatment than in cases treated early in the infection.

A period of "depression" was noted in most groups 70 to 80 days after infection when there was a fall in body weight and relapses; deaths and photosensitisation also occurred.

The toxicity of dimidium bromide for bovines is briefly discussed.

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FIG. 3. Bovine 5247 on the 18th week after infection and 15 weeks approximately after treatment, showing the areas of skin most affected by photosensitisation.

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THE DEVELOPMENT OF HELMINTHIASIS IN
THOROUGHBRED FOALS

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INTRODUCTION

The great importance of parasitic worms to breeders of horses throughout the world is well recognised and the continual effort which is required in order to keep the infestation of strongyloid nematodes down to a minimum is one of the principal worries of all who are intimately concerned in the rearing and training of thoroughbred horses.

The great complexity of the causal relationships of these parasites with disease in the horse, is however not generally appreciated, but becomes apparent when it is pointed out that some 40 to 50 different species of strongyloid worm are concerned. Any combination of any number of these species may be present together in one and the same horse. Le Roux (1924) in a series of *post-mortem* examinations on working horses in Edinburgh and London, found representatives of some 33 species, while Robertson (1939) found 21 species when examining four yearling Shetland ponies which died when brought over from Orkney to the mainland. No effort was made to enumerate the individual parasites, but there is also a very large scope for variation in that respect. Workers at the Helminthological Institute in Moscow made laborious *post-mortem* examinations on two moderately infested horses and found 52,000 and 200,000 individual worms. They also found 32,900 worms of 27 different species in the colon of a two year old stallion, the number of any one species varying from one to 12,159 individuals (Bederke, 1931). The different species vary from one another in their life histories, in the course of their migrations through the body of the horse, in the time taken for their complete development and in their pathogenic effects. The field for investigation is thus seen to be a very wide one with many particular problems of its own, in addition to the more general problems of helminthology.

In beginning to make a study of the strongylosis position with regard to the rearing and management of bloodstock, it was considered wise to carry out first of all some kind of a survey and to begin with observations on the development of worm infestation in thoroughbred foals reared under the ordinary conditions of stud management.* Arrangements were therefore made with seven

* This work was carried out at the Ministry of Agriculture and Fisheries Veterinary Laboratory, while in receipt of a Research Fellowship under the Veterinary Educational Trust.

different studs to submit weekly samples of faeces from some of the foals born there, and a series of egg counts and larval differentiations has been made on 26 foals from about four weeks onwards to at least six months. In some cases it was possible to continue examining samples till the foals were over a year old. A few faecal samples from the dams were also examined in order to find out whether any relationship existed between the infection acquired by the foal and that carried by the dam.

LABORATORY TECHNIQUE

The samples were collected in 2-oz. bottles, and were despatched to the laboratory on the same day. Egg counts were then made using a modification of the Stoll technique, or by the Clayton-Lane direct centrifugal flotation technique in those samples in which the Stoll count was negative, *i.e.*, where the number of eggs proved to be less than 100 per gram.

A faeces culture made from each sample on the day of its arrival at the laboratory, was kept at either 26° C. for seven days, or at room temperature for 12 days when, as frequently happened, it was not possible to provide the necessary incubator accommodation. At the end of this period the larvae were separated from the faeces and examined under the microscope in order to obtain some more detailed information on the species of red-worm and their proportional representation among the eggs that had been counted in each of the samples. The ordinary Baermann apparatus was used for this purpose at first, but the process of cleaning the glass funnels between cultures took so much time that it was eventually decided to revert to a more primitive technique. The sample was tied up in a square of sterile muslin and allowed to float in a pint measure full of water for 24 hours. After removing the culture, the water was allowed to sediment for a further 24 hours. The supernatant fluid was then syphoned off and the larvae obtained in a small quantity of water. This method was found to be perfectly satisfactory, and although the larvae were not quite so clean as those obtained by means of the Baermann apparatus, the small amount of debris did not interfere with their differentiation in any way.

The suspension was next transferred to a watch glass where it could be suitably diluted if necessary and after evenly distributing the larvae by bubbling air through the suspension, a few drops were taken out, placed on a slide and covered with a $1\frac{1}{2} \times \frac{7}{8}$ inches cover slip. When only a very few larvae had been recovered, these were all picked out with a fine pipette under the dissecting microscope, transferred to a slide and then systematically examined from end to end, identifying the species of every one as far as the knowledge of their morphology would permit. Further slide preparations were made, if necessary, until one hundred larvae had been examined and placed either in their specific or generic group. Obviously, it was not permissible by this method to pass over any larvae that could not be easily identified in the course of the examination. It was necessary, however, to reach a decision on the identity of each and every successive individual until the predetermined number had been completed; otherwise the observations on the proportional representation of species in the egg count might have been materially altered. The killing of the larvae by the smallest amount of heat necessary was, therefore, most important and trials of various methods ultimately led to the use of the heat of the resistance

coil of the microscope lamp. The glass slide with living larvae in water under the cover slip was allowed to rest on the metal grating over this coil. Registration of the temperature of this site, as nearly as could be secured by means of an ordinary mercury thermometer, showed it to be somewhere in the region of 180° C. About 20 seconds was found to be the minimum time in which the most resistant larva was killed, but as the time varied slightly with the age of the larvae and the amount of water under the cover-slip, it was necessary to control the process by intermittent microscopical examination in order to be certain that the larvae were killed without over-heating.

A simpler method of killing larvae by adding dilute Gram's Iodine, as described by Whitlock (1942) was discarded as it was found to cause some distortion of the internal structure.

IDENTIFICATION OF THE LARVAE

The third stage larvae of the equine strongyles are not all well known, but published descriptions enable a differentiation of the following genera and species to be made :—

Strongylus vulgaris, *Strongylus edentatus*, and *Strongylus equinus* (Wetzel 1930), *Poteriostomum* spp. *Trichonema* * spp. and *Gyaloccephalus capitatus* (Lucker, 1934, 1935, 1936, and 1938) and *Triodontophorus* spp. (Petrov and Gagarin, 1937). The infective larva of *Trichostrongylus axei* has been described in papers on the larval stages of sheep nematodes (Monnig, 1931 : Gordon, 1933) and that of *Strongyloides westeri* by Frischbier (1930). No attempt was made to differentiate the species of *Trichonema*, although some of these have been described, nor were any measurements made except in a few doubtful cases. Larvae were differentiated entirely from the following characteristics :—size and shape, comparative lengths of oesophagus and tail of sheath to total length, number and shape of intestinal cells. It was essential to examine the larvae within a few weeks of making the culture, as although some retained all their characteristics when kept as long as four months in cold storage, in the majority of cases the intestinal cells became indistinct after a few weeks and could not be counted. Although the larvae of *Strongylus* spp. can quite easily be distinguished without counting the cells, those of the smaller worms belonging to the other genera can only be differentiated with certainty if the intestinal cells are clearly visible.

The following key to the differentiation of the infective larvae was worked out for use in these observations :—

Trichonema spp. Gut comprising eight clearly defined triangular cells. Very variable in size and shape. Oesophagus and tail of sheath comparatively long.

* The generic name *Trichonema* is used throughout this paper to describe all the species of small cylicostomes. Although it is realised that the American authors have a good case for rejecting this name on the grounds that the species *Trichonema arcuata* as described by Cobbold (1874) has never been validated, it is considered best to keep to the name hitherto used by the majority of European authors, pending a decision on this troublesome question by the International Commission on Zoological Nomenclature.

Gyalocephalus capitatus. Gut comprising 12 clearly defined rather elongated cells. Larva short and broad. Oesophagus and tail of sheath of medium length. Small bulbous process on end of larval tail.

Poteriostomum spp. Gut comprising 16 clearly defined slightly rectangular cells. Outer sheath wrinkled and with long tail. Broad larva of medium length.

Trichostrongylus axei. Gut as *Poteriostomum* spp. Outer sheath smooth, without whip-like tail, long narrow larva with characteristic kink near tip of sheath and knob on larval tail.

Strongylus edentatus. Gut comprising 18 to 20 cells. Cells elongated and ill-defined. Small, thin larva with short oesophagus. Tail of larva bluntly rounded and tail of sheath short and tapering gradually over it without constriction.

Triodontophorus spp. Gut as *Strongylus edentatus*. Cells well defined and rectangular. Broad larva of medium length with long oesophagus, and tail of sheath.

Strongylus vulgaris. Gut comprising 28 to 32 clearly defined, dark, rectangular cells. Very long broad larva with short oesophagus and tail of sheath.

Strongyloides westeri. Intestinal cells not clearly delineated. Oesophagus nearly half length of body. Small thin larva without protective sheath or long tail. End of larva appears notched in lateral view, due to three small processes, two subventral and one dorsal.

The larva of *Strongylus equinus* was never seen in any of these samples, but can be easily distinguished as it is very long and thin with 16 ill-defined cells, short oesophagus and tail of sheath and a small trilobed process on the larval tail.

A few larvae did not answer to any known description and were put down as unidentified. The most common was a very large larva, closely resembling that of *Strongylus vulgaris* in size and shape, but having only 16 very large and well-defined triangular cells. The measurements are given below with those of an average *S. vulgaris* larva for comparison.

					Unidentified larva	<i>S. vulgaris</i>
Length of sheath	...	...	...	...	1.080 μ	900 μ
Length of larval body	...	...	...	...	746 μ	635 μ
Width of sheath	...	...	...	...	37.5 μ	28 μ
Length of oesophagus	...	...	...	...	184 μ	143 μ
Length of intestines	...	...	...	...	461 μ	413 μ
Distance from excretory pore to anterior end of body	...	...	...	...	139 μ	103 μ

This larva was found in samples from one foal at Stud A and in five out of six foals at Stud F, and its earliest appearance was at

22 weeks in foal 15. Whether it belongs to the genus *Craterostomum* or represents the infective larva of *Oesophagodontus robustus* can only be conjectured.

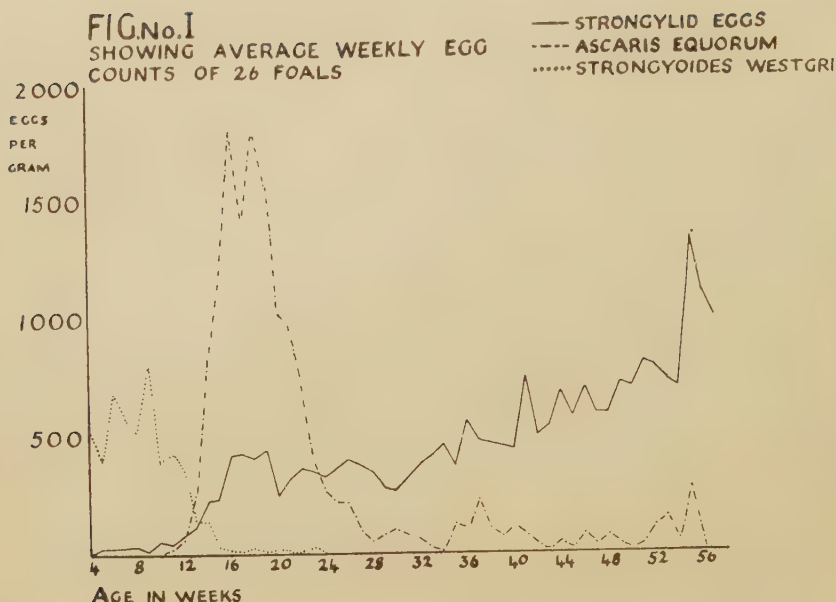
DATA OBTAINED FROM EGG COUNTS AND FAECAL CULTURES

The detailed results of the egg counts and larval differentiations are too numerous to be included in this paper, but all the figures are kept at the Laboratory of the Ministry of Agriculture and Fisheries,

TABLE I
SHOWING THE AVERAGE EGG COUNT OF ALL 26 FOALS IN EGGS OR LARVAE
PER GRAMME OF FAECES

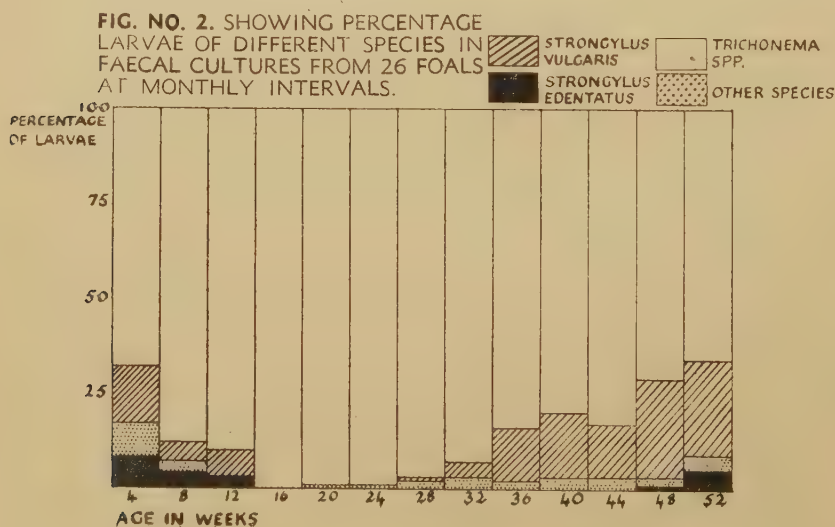
Age in weeks	...	4	5	6	7	8	9	10	11	12
Strongylid eggs	...	18	22	21	28	31	16	53	39	75
<i>Ascaris equorum</i>	...	—	—	—	—	—	1	—	20	62
<i>Strongyloides westeri</i>	...	536	392	682	582	524	818	400	439	362
Age in weeks	...	13	14	15	16	17	18	19	20	21
Strongylid eggs	...	105	232	234	427	426	404	435	247	315
<i>Ascaris equorum</i>	...	280	813	1,169	1,808	1,408	1,800	1,572	1,035	978
<i>Strongyloides westeri</i>	...	147	171	31	24	6	12	6	18	6
Age in weeks	...	22	23	24	25	26	27	28	29	30
Strongylid eggs	...	354	348	327	361	396	372	347	278	273
<i>Ascaris equorum</i>	...	732	400	260	212	213	90	49	71	100
<i>Strongyloides westeri</i>	...	6	18	—	—	—	—	—	—	—
Age in weeks	...	31	32	33	34	35	36	37	38	39
Strongylid eggs	...	318	380	412	466	369	559	481	473	400
<i>Ascaris equorum</i>	...	79	67	24	8	122	111	233	100	60
<i>Strongyloides westeri</i>	...	6	—	—	—	—	—	—	—	—
Age in weeks	...	40	41	42	43	44	45	46	47	48
Strongylid eggs	...	393	747	500	527	686	571	693	593	593
<i>Ascaris equorum</i>	...	107	93	47	7	40	14	79	29	64
<i>Strongyloides westeri</i>	...	—	—	—	—	—	—	—	—	—
Age in weeks	...	49	50	51	52	53	54	55	56	57
Strongylid eggs	...	721	700	816	790	725	700	1,340	1,100	1,000
<i>Ascaris equorum</i>	...	29	8	27	110	150	50	340	—	—
<i>Strongyloides westeri</i>	...	—	—	—	—	—	—	—	—	—

Weybridge, and are available for reference to anyone who may wish to consult them. The weekly counts are summarised in Table I, which gives the average for all foals examined. The eggs fall into three groups:—(1) The strongyloid eggs, including those of *Strongylus vulgaris*, *Strongylus edentatus*, *Trichonema* spp., *Poteriostomum* spp., *Gyalcephalus capitatus*, *Triodontophorus* spp., and the eggs of the trichostrongylid nematode *Trichostrongylus axei*;



(2) the eggs of *Strongyloides westeri*; (3) the eggs of *Ascaris equorum*.

Eggs of *Oxyuris equi* were found in the faeces of one mare and her foal, but it was not possible to get any further samples, and she is not included in the tables. The point is recorded only as a matter of interest, since the eggs of this parasite are not generally passed in the faeces.



It will be seen from the table that the eggs of the various species and groups of species of worms begin to show themselves in the foal faeces at different ages, a point illustrated graphically in Fig. 1 which shows the average figures for the whole group of 26.

TABLE II
SHOWING THE AVERAGE WEEKLY PERCENTAGE OF DIFFERENT LARVAE IN FAECAL CULTURES FROM 26 FOALS

Age in weeks ...	4	5	6	7	8	9	10	11	12
<i>Trichonema</i> spp. ...	68	78	89	86	88	88	91	94	91
<i>Strongylus vulgaris</i> ...	15	17	6	10	5	7	5	2	7
<i>Strongylus edentatus</i>	8	5	5	4	4	2	2	4	2
Other species ...	9	—	—	—	3	3	2	—	—
Age in weeks ...	13	14	15	16	17	18	19	20	21
<i>Trichonema</i> spp. ...	98	99	100	99	99	99	99	99	99
<i>Strongylus vulgaris</i> ...	1	1	—	—	—	—	—	—	—
<i>Strongylus edentatus</i>	1	—	—	—	—	—	—	—	—
Other species ...	—	—	—	1	1	1	1	1	1
Age in weeks ...	22	23	24	25	26	27	28	29	30
<i>Trichonema</i> spp. ...	98	98	98	99	97	97	97	96	98
<i>Strongylus vulgaris</i> ...	—	—	—	—	1	1	1	2	1
<i>Strongylus edentatus</i>	—	—	—	—	—	—	—	—	—
Other species ...	2	2	2	1	2	2	2	2	1
Age in weeks ...	31	32	33	34	35	36	37	38	39
<i>Trichonema</i> spp. ...	96	93	91	91	86	84	83	78	76
<i>Strongylus vulgaris</i> ...	3	4	7	7	12	14	15	17	22
<i>Strongylus edentatus</i>	—	—	—	—	—	—	—	—	—
Other species ...	1	3	2	2	2	2	2	5	2
Age in weeks ...	40	41	42	43	44	45	46	47	48
<i>Trichonema</i> spp. ...	80	81	81	80	83	78	75	74	71
<i>Strongylus vulgaris</i> ...	17	17	16	17	14	20	22	22	26
<i>Strongylus edentatus</i>	—	—	—	—	—	—	—	1	1
Other species ...	3	2	3	3	3	2	3	1	2
Age in weeks ...	49	50	51	52	53	54	55	56	
<i>Trichonema</i> spp. ...	71	73	66	71	74	75	81	79	
<i>Strongylus vulgaris</i> ...	24	21	25	25	22	20	13	15	
<i>Strongylus edentatus</i>	2	3	5	1	1	2	2	1	
Other species ...	3	3	4	3	3	3	4	5	

The differential larval counts from the 26 foals are summarised in Table II and illustrated in Fig. 2, while Table III indicates the approximate age in weeks after foaling at which eggs or larvae of the different species first appeared in the faeces. Table V shows a comparison between the final differentiation of 100 larvae in some of the older foals with that of their dams before weaning.

TABLE III

SHOWING TIME OF FIRST APPEARANCE IN THE FAECES OF THE EGGS OR LARVAE OF THE DIFFERENT SPECIES OF PARASITIC WORMS

Species and number of foals in which each was observed	Age of foals in weeks		
	Average age of first appearance	Earliest age of first appearance	Latest age of first appearance
<i>Strongyloides westeri</i> (17 foals) ...	5.7	4	11
<i>Ascaris equorum</i> (26 foals) ...	13.23	9	16
<i>Trichonema</i> spp. (26 foals) ...	14	12	17
<i>Trichostrongylus axei</i> (17 foals)...	17.76	4	49
<i>Poteriostomum</i> spp. (25 foals) ...	20	15	32
<i>Gyalocephalus capitatus</i> (13 foals)	28	17	50
<i>Strongylus vulgaris</i> (19 foals) ...	29.74	25	36
<i>Triodontophorus</i> spp. (16 foals)...	31.75	18	46
<i>Strongylus edentatus</i> (8 foals) ...	50.87	40	55

N.B.—In the case of *Trichonema* spp. a marked rise in egg count together with 100 per cent. *Trichonema* larvae in cultures is taken to indicate commencement of genuine infection.

TABLE V

A COMPARISON BETWEEN THE REDWORM INFESTATION OF THE INDIVIDUAL MARES AND THEIR FOALS AS SHOWN BY A DIFFERENTIATION OF 100 LARVAE FROM FAECAL CULTURES AT THE 52ND WEEK OF THE FOAL'S LIFE

Species	No. 1		No. 2		No. 15		No. 16		No. 17	
	Mare	Foal	Mare	Foal	Mare	Foal	Mare	Foal	Mare	Foal
<i>Trichonema</i> spp. ...	58	96	92	98	86	65	66	40	81	64
<i>Poteriostomum</i> spp. ...	—	3	—	1	1	1	—	1	—	3
<i>Triodontophorus</i> spp. ...	—	—	—	—	—	1	—	—	—	—
<i>Trichostrongylus axei</i> ...	—	—	—	—	2	—	1	—	1	1
<i>Strongylus vulgaris</i> ...	32	1	3	1	10	33	31	59	13	31
<i>Strongylus edentatus</i> ...	10	—	5	—	1	—	2	—	5	1
Species	No. 18		No. 19		No. 20		No. 21		No. 26	
	Mare	Foal	Mare	Foal	Mare	Foal	Mare	Foal	Mare	Foal
<i>Trichonema</i> spp. ...	83	75	14	70	64	58	92	80	93	68
<i>Poteriostomum</i> spp. ...	—	10	—	—	—	5	—	2	—	—
<i>Triodontophorus</i> spp. ...	—	—	—	—	—	—	—	—	—	—
<i>Trichostrongylus axei</i> ...	—	—	—	—	—	—	—	—	—	—
<i>Strongylus vulgaris</i> ...	7	15	81	29	28	34	6	18	6	32
<i>Strongylus edentatus</i> ...	10	—	5	1	8	3	2	—	1	—

RESULTS

A study of Tables I, II and III and of the Figs. 1 and 2, will show something of the order of occurrences in this series of observations, the different ages at which the presence of the various species was first observed in foals, their rate of increase and in some cases their rate of disappearance. It is proposed to deal with these in detail under the headings of the various species of worms concerned and subsequently to discuss any factors which appeared to influence the development of the infestation.

The Strongyles or Red Worms

This large and important group constitutes many species, all of which produce the general type of strongyloid egg and cannot be differentiated from one another by that means, nor from the trichostrongylid worm, *Trichostrongylus axei* which does not belong to this group. The morphological characters of the third stage larvae made it possible, however, to recognise some of these species.

The very early appearance of strongyloid eggs came as a surprise and was at first considered to be suggestive of a prenatal infection. When it was found however that every one of the 26 foals was passing numbers of eggs, including those of *Trichonema* spp., which do not migrate beyond the intestinal wall and could not conceivably infect the foetus in utero, some other explanation was sought. The suggestion of Wetzel (1942) that strongyloid eggs passed by foals under six weeks old are referable to coprophagia has been confirmed. As young foals are often seen nibbling at the freshly passed faeces of their dams it must be concluded that this is a normal habit and that it occurs to a much greater extent than was hitherto supposed. Considerable quantities must be ingested in many cases. For example, the egg count of foal 11 at five weeks was 100 e.p.g., while that of its dam for the same week was 1,200 e.p.g. If the foal was passing per day 2 lb. of faeces with a total of approximately 90,800 eggs it must have eaten about $2\frac{1}{2}$ oz. of the dam's faeces in order to produce the count of 100 e.p.g.

It will be noticed that the egg count in the foals shows a slight tendency to fall from the sixth to tenth weeks, which may be explained either by a diminished consumption of the mare's faeces, or by an increase in the bulk of the foal's own faeces. A comparison between the egg counts of mares and foals in the first few weeks of life shows a definite correlation between the extent of infection in the mare and the number of eggs passed by the foal. In mare 22 with an average egg count of 110 e.p.g., the average count in the foal from five to 13 weeks was about 5 e.p.g., while foal 8 whose dam averaged 1,840 e.p.g. showed an average count of 65.5 e.p.g. in the same period. The count of the foal was usually found to be between 1 per cent. and 10 per cent. of that of its dam, but this proportion varied considerably from foal to foal and from week to week and no constant ratio could be observed.

Further evidence as to the source of the strongyloid eggs passed by foals under three months, was obtained from the larval cultures. Eggs swallowed by the foal do not undergo any further development, presumably owing to lack of oxygen, but the majority retain their viability and are able to hatch out and develop into infective larvae when passed out in the foal's faeces. A perusal of the differential larval counts of mares and foals in the first few weeks shows that the type of infection carried by the mare is reflected in the foal's faeces, all the different species being represented in very similar proportions.

Evidence of genuine infection with redworms did not appear until the foal was 12 to 14 weeks old, when there was usually a sharp rise in the egg count. At the same time the larval cultures began to show a predominance of *Trichonema* spp. The prepatent period of these small strongyles is believed to be eight to 12 weeks (Wetzel, 1942) and infection must therefore take place in the first week or two, presumably through picking up infective larvae in the box. The egg count continues to rise for four to six weeks, after which it tends to remain level or decline slightly for some months. During this period (16 to 26 weeks) the eggs of another group of redworms begins to appear, namely those of *Gyalocephalus capitatus*, *Poteriostomum* spp. and *Triodontophorus* spp. The infective larvae of these species are seldom seen in faecal cultures from adult horses, but frequently occur in considerable numbers in foals up to one year. It is possible that older horses develop an age immunity to these species, or it may be that they are poor egg producers and that their infective larvae are swamped by those of the more prolific *Trichonema* and *Strongylus* spp. The life histories of these worms are not well known, but are believed to be similar to that of *Trichonema*. Ortlepp (1925) concludes that *Triodontophorus* spp. do not enter the mucous membrane, but develop to maturity in the lumen of the large intestine, although there is no experimental evidence to support this theory.

The infective larvae of *Poteriostomum* spp. appeared regularly in 25 out of the 26 foals, in some cases (foals 18 and 20) accounting for up to 10 per cent. of the total infection. *Gyalocephalus capitatus* was present in 13 and *Triodontophorus* in 16 of the 26 foals.

From the age of 26 weeks onwards the larvae of *Strongylus vulgaris* begin to appear and increase in numbers. This corresponds with the work of Wetzel (1942) who gave the prepatent period of *S. vulgaris* as six and a half months. The marked rise in the strongyloid egg count from about 30 weeks onwards is due in most cases to the eggs laid by this parasite when it reaches maturity. *S. vulgaris* was only missing in those foals from which it was not possible to obtain samples after weaning; in all the older foals however it occurred constantly in considerable numbers constituting in some cases as much as 50 to 60 per cent. of the total infection. It is remarkable that the percentage of *S. vulgaris* was highest in mares

and foals from well-managed studs (F and G) where the egg counts were comparatively low and it seems reasonable to conclude that this parasite is particularly resistant to the usual methods of anthelmintic treatment and pasture management, so that as the general level of infection drops, the proportion of *S. vulgaris* tends to rise. As it is considered to be the most pathogenic of all the redworms because of the damage done to the arterial walls by the larvae in the course of their migrations, the appearance of this species must be regarded as a serious problem.

The last of the redworms to appear was *Strongylus edentatus* which with one exception, did not begin to lay eggs until the 50th to 55th week. *S. edentatus* is among the most common of the equine strongyles and was present in every one of the mares, so that it seems reasonable to assume that, as with the other species, infection with this parasite was acquired by the foal in the first week of life. Of the 13 foals from which samples were received during the 50-week period or longer, only eight were passing eggs of *S. edentatus* when the sampling was discontinued. In all the other cases a careful search was made through large numbers of larvae (after the usual 100 individuals had been differentiated) in order to make sure that they were absent.

As the initial appearance of the larvae was followed by a gradual increase in numbers, it is concluded that the worms were just beginning to mature in these animals and to lay eggs. This is well illustrated by foals 19 and 20, where the percentage of *Strongylus edentatus* larvae in cultures were as follows :—

Weeks after foaling	Foal 19	Foal 20.
50	None	None
51	None	1 per cent.
52	1 per cent.	3 per cent.
53	1 per cent.	6 per cent.
54	5 per cent.	4 per cent.
55	—	5 per cent.

The prepatent period therefore appears to be 50 to 52 weeks, a figure which is considerably at variance with that of three months given by Martin (1910) and hitherto undisputed.

In foal 23, eggs of *S. edentatus* first appeared in the faeces at 40 weeks and continued to increase until at 51 weeks they represented 45 per cent. of the total infection. With the small amount of evidence available it is impossible to reach any justifiable conclusion about this exceptional case, but it would appear to indicate that the parasite may complete its development within 40 weeks of infection. The true explanation may however be in the occurrence of prenatal infection. Immature forms of *S. edentatus* can be found all over the peritoneal cavity and there is no reason to suppose that some do not penetrate the foetus where they find favourable conditions for further development. Prenatal infection has been

definitely proved to occur with *Ancylostoma caninum*, in dogs (Foster 1932 and 1935) but experimental proof of its occurrence with horse strongyles is still lacking.

The Stomach Worm, Trichostrongylus axei

This worm was present in 17 foals from six out of the seven studs although it did not occur in any of the three from Stud D. It is noteworthy that this was the only stud where no cattle had been grazed during the previous year on the pastures used for mares and foals. This bears out observations made by Foster (1937) and others to the effect that *Trichostrongylus axei* can only maintain itself in the horse when the latter is kept in contact with cattle or sheep.

The prepatent period of the stomach worm is 21 days and in some cases infective *T. axei* larvae were present in the foals' faeces at four to six weeks, even when the mares appeared to be free from infection (foals 14 and 18). Where the mares were infected with *T. axei* (3 and 4) it was impossible to distinguish a genuine infection in the foals from eggs picked up when eating dung. *Trichostrongylus axei* is known to produce comparatively few eggs per female (Gordon, 1933) so that the infection carried by mare 3 (up to 88 e.p.g.) may be regarded as quite heavy. Although cases of equine trichostrongylosis have been described by Christensen (1945) and other authors, the pathogenicity of this parasite in the horse has not been definitely established and no evidence of disease due to stomach worm infestation was obtained in this series of observations.

Strongyloides westeri

Seventeen of the 26 foals from six studs showed an infection with *Strongyloides westeri*. Embryonated eggs appeared in the faeces at four to eight weeks and counts up to 7,600 e.p.g. were noted at eight to nine weeks. Salisbury, Baker and Britton (1940) found that the first appearance of *Strongyloides* eggs in the faeces at 22 days was often associated with a transient attack of diarrhoea, but none of the foals in the present series of observations were affected in this way. The prepatent period for *Strongyloides* is about 15 to 21 days (de Blieck and Baudet, 1921) so that possibly some of the foals had already passed this stage before material was first collected. In any case, it is usual for foals to scour when their dam first comes into oestrus nine to 28 days after foaling so that the presence of *Strongyloides* is not necessarily responsible for this condition. None of the infected thoroughbred foals showed any sign of diarrhoea or unthriftiness at any time: moreover all acquired an immunity and threw off their *Strongyloides* within a few weeks. The egg count began to fall steadily from about the tenth week and all except one foal were free of the parasite at 15 to 23 weeks.

Cases of supposed *Strongyloidosis* in foals have been reported

from time to time, most of which have recovered spontaneously in a few weeks. Davidson (1943) describes a fatal case in a three and a half months old Clydesdale. The symptoms were persistent scouring and anaemia, with a count of 10,000 eggs of *Strongyloides westeri* per gram. The foal finally died after treatment with *Liquor arsenicalis* and 85,000 adult worms were found in the small intestine, but no inflammatory changes were seen. In view of the high egg counts noted in perfectly healthy thoroughbred foals and the absence of any lesions caused by the parasite, it seems unlikely that *Strongyloides westeri* was the cause of death in this case.

In connexion with the pathogenicity of this parasite, it may be of interest to record the case of an experimental foal (169) kept in a loose box at the Laboratory at Weybridge from 24 hours old and never allowed outside. A faecal examination made at 22 days showed that it was passing 50,000 eggs of *Strongyloides westeri* per gram. The foal was in excellent

TABLE IV
COURSE OF INFECTION WITH *Strongyloides westeri* IN EXPERIMENTAL FOAL 169

Age in weeks	No. of infective larvae applied to the skin	Eggs per gramme
3		50,000
4		46,000
5		39,000
6		45,000
7		36,000
8	90,000	22,000
9	20,000	6,300
10	80,000	3,500
11	20,000	4,100
12	450,000	3,700
13	45,000	2,400
14		500
15		22

condition and never scoured. The egg count remained at a high level till the foal was seven weeks old when it began to decline. Attempts were then made to reinforce the infection. Cultures were made of the foal's faeces. Infective larvae separated from the faeces after three day's incubation, were counted and applied in a little water to the skin. In spite of the repeated application of enormous numbers at weekly intervals, the egg count continued to diminish steadily. When the foal died from an acute septicaemic condition of independent origin at 15 weeks, the egg count was only 22 e.p.g., and on *post-mortem* examination only 51 adult *Strongyloides westeri* were recovered from the small intestine. Although this probably did not represent the full extent of the infection, no attempt having been made to scrape the mucous membrane in order to secure worms embedded in the surface layers, it

is clear that the foal had acquired a strong immunity and was able to resist super-infection. Details of the egg counts and numbers of larvae used are given in Table IV.

The result of this experiment was in accordance with the work of Sandground (1928) on the course of infections with *Strongyloides stercoralis* in dogs and cats. He found that the animals were not susceptible to super-infection once immunity had started to develop. He also showed that the development of immunity was absolutely dependent on a previous infection and had nothing to do with the age of the animal. In order to find out if possible whether this was the case with *Strongyloides westeri* an attempt was made to infect the dam of foal 169, a six-year old pony mare, by repeated application of infective larvae to the skin. These attempts failed completely, no eggs appearing in the faeces over a period of ten weeks, in spite of a total application of 705,000 infective larvae. It can only be assumed that the mare had been infected early in life and had thus acquired a lasting immunity.

In both mare and foal the larvae seemed to penetrate the skin as shown by the intense irritation and subsequent roughening and thickening of the site, further confirming the observations of Sandground (1928) who found that neither the skin nor the blood stream constituted a barrier in immune animals and that larvae were able to reach the intestine but not to mature there.

The most interesting feature about *Strongyloides westeri* is the way in which a massive infection is able to develop at a very early age even in housed animals. The adult females are not prolific egg layers, seldom containing more than eight to 12 mature eggs in the uterus at once, so that the count of 50,000 e.p.g. in the case of foal 169 must have represented an enormous numbers of worms. At the same time no eggs could be demonstrated in the faeces of the mare, nor did any of the thoroughbred mares show any signs of infection with *Strongyloides* even by flotation methods. The fact that foal 169 became so heavily infected although never out to grass and that some thoroughbred foals acquired heavy infection while others from the same stud and grazing the same pasture remained absolutely free, suggests that the boxes rather than the pasture were the source of infection. Most foaling boxes are submitted to a very rigorous cleansing and disinfection before use. In the case of Stud C, although the glazed tile walls and concrete floors of the foaling boxes were scrubbed out with hot caustic soda solution and had been empty for nine months before use, both the foals from which samples were examined, acquired an infection. There is, of course, a possibility that these foals may have become infected in utero. Dr. Andrews, of Tifton, Georgia, reports in a personal communication that he found adult *Strongyloides* females in a piglet only two days old and is convinced that prenatal infection with *Strongyloides ransomi* does occur. Infective larvae of *S. westeri* which penetrate the skin or are taken in by mouth may not be

able to reach maturity in the mare, but those which reach the foetus in the course of their migrations may find favourable conditions for further development there. Such cases are probably exceptional however, and some other explanation for the persistent survival of this parasite must be sought. It can only be concluded that either the infective larvae, or the free living adult stages of *Strongyloides* are much more resistant to external influences than has been hitherto supposed, and a lot of work remains to be done to solve this interesting problem.

Ascaris equorum

Every one of the 26 foals acquired an infection with *Ascaris*, eggs first appearing in the faeces in most cases at 12 to 13 weeks. Two foals (6 and 16) were passing eggs at nine weeks. This is difficult to reconcile with the 81 day period necessary for the development of *Ascaris* in the foal as given by Wetzell and Einigk (1938), but might conceivably be the result of prenatal infection. This has been experimentally proved to take place in the case of *Belascaris* in the dog (Augustine, 1927; Foster, 1935) and observations strongly suggestive of its occurrence in the case of *Ascaris vitulorum* in calves have been made by Griffiths (1922) and Macfie (1922). Salisbury, Baker and Britton (1940) reported a case of a foal which consistently passed *Ascaris* eggs from the 22nd day and ascribe this to prenatal infection. If this explanation is correct development must still have been very rapid, as it has been established (Augustine, 1927) that the growth of parasitic larvae in the foetus is retarded and that they remain in the liver until parturition has taken place, when they are able to complete their migrations and reach maturity. Although the larvae of *Ascaris equorum* fulfil the conditions laid down by Foster (1935) as essential to the establishment of prenatal infection (blood stream migration and tendency of the larvae to penetrate tissue barriers) further experimental work on the prepatent period of *Ascaris* in the horse would be necessary before coming to any final conclusion.

The count of *Ascaris* eggs rises very sharply and reaches a much higher level than the strongyle egg count in a very short time. This does not necessarily indicate a heavy infection, as ascarids are very prolific egg producers and the presence of a single mature female would probably lead to a count of several hundred e.p.g. The peak of egg production was reached at 16 to 18 weeks, after which it declined steadily and many foals ceased to pass *Ascaris* eggs from about six months onwards. In others the immunity was not absolute and they continued to pass a few eggs at intervals. Examination of one or more sample from each mare by a flotation technique failed to show any *Ascaris* eggs and it seems probable therefore that it is the foals and yearlings which keep infection going on a stud and that a fairly complete immunity is acquired later in life.

Most of the foals at Stud C and F were coughing and showed a dry, sticky nasal discharge at weaning time, and this was attributed by the owners to *Ascaris* infection.

Dictyocaulus arnfieldi

Careful search was made for the characteristic larvae of the horse lungworm described by Wetzel (1938) when making egg counts and examining larval cultures, but none were seen.

DISCUSSION

Correlation of infection of foals with that of their dams. A perusal of Table V will show that very little correlation existed between the type of infection carried by mares and that finally acquired by their foals. It is said that some mares have a natural resistance to worm infection and that they are able to transmit this resistance to their foals (Salisbury, Baker and Britton, 1940) but no reliable evidence of this could be obtained in such a limited series of observations. One mare (22) had a remarkably low level of infection (average 110 e.p.g.), but this was ascribed by her owner to the fact that she had only just been brought over from a large farm in Ireland where very few horses were kept. Her foal showed no special resistance to infection and had a count of 1,200 at 34 weeks.

It will be noted that the older foals nearly all carry a much higher percentage of the large strongyles, particularly *S. vulgaris*, than their dams and this may be due to a greater susceptibility of young animals to these parasites.

Redworm counts in the mares remain at a fairly constant level, although there is a distinct seasonal tendency to rise in the late summer, presumably as a result of the increased intake of infective larvae in the spring. The egg count in the foals on the other hand is continually rising as the varying species mature and start to lay eggs and a fairly constant level of infection is probably not reached until two to three years old.

It would have been enlightening to make many more comparisons than were done, but time would not allow as the amount of work was too great.

Correlation of infestations of foal with date of birth. Examination of the detailed egg counts fails to show any significant difference in the rate at which infection was acquired by foals born in January (1 and 18) and by late foals (22 and 28). It was expected that late foals would pick up an infection more rapidly owing to the favourable conditions for the development of infective larvae obtaining in the late spring, but this did not appear to be the case. Some authors believe that eggs cannot hatch and develop outside during the winter months even in England and if this is so, the early foals must have acquired their infection either from larvae maturing in the autumn which had been able to survive the winter, or from

larvae picked up in the box from contaminated straw, walls, mangers, etc. The latter explanation seems the most probable as pastures used for foals are usually left vacant for at least six months and the work of Taylor (1938) has shown that the mortality rate of larvae on such a pasture in the winter months would probably be very high.

Correlation of infestation of foal with the stud. The rate at which infections developed in foals appeared to be largely dependent on the management of the stud on which they were reared. On some studs the general level of infestation in mares and foals was low, notably Stud A. The average egg count of two mares (1 and 2) from this stud was 600 and 610 e.p.g. and their foals maintained a low level of infection (less than 1,000 e.p.g.) till they were nearly a year old. Stud A was particularly well managed, ample grass being available: cattle were run on the paddocks throughout the winter and spring and there was enough labour to pick up the droppings in every paddock at least twice a week.

Stud C. on the other hand, was very short of labour and had been overstocked with horses for many years. No attempt was made to collect droppings, which were constantly scattered by rooks. Many of the paddocks were badly drained and parts were under water in the winter and spring. Conditions therefore were very favourable to the development of redworm infestation and a few years ago when mares and foals were allowed to run out night and day in the summer, one foal had died of a ruptured verminous aneurism at six months old. The average counts of the three mares (7, 8 and 9) from this stud were 2,210, 1,840 and 810 e.p.g. respectively. Mare 9 had been recently bought and had not been on the stud many months. Foals 7 and 9 showed an egg count of over 1,000 e.p.g. at 16 weeks and foal 7, a January foal, subsequently showed consistently high counts of 2,000 e.p.g. and more. Foals 8 and 9 did not reach such a high level, but both were in very poor condition when weaned. Unfortunately, these foals were sold in December and it was not possible to continue examining samples from them. It is probable, however, that their counts as yearlings would have been very high.

The rest of the studs were intermediate and may be described as comparatively clean. Much thought had been given to the problem of redworms and grazing hygiene was practised as far as wartime conditions would allow. The average egg counts of the mares from these studs were seldom over 1,000 e.p.g. and most of the foals did not develop a heavy infestation. It will be seen, therefore, that there is a definite correlation between stud management and the extent of infestation with redworms.

This does not appear to be the case with regard to *Ascaris* infection, however, which occurred to an equal extent on every stud and on which rotational grazing and the removal of droppings appeared to have little effect. The thick shelled eggs of *Ascaris* are

able to survive for long periods on pastures or adhering to walls and floors of boxes and may be less susceptible to external influences than are the infective larvae of the strongyloid worms.

Infestation with this parasite cannot therefore be so readily controlled by pasture hygiene and the only effective measure against it would appear to be the use of absolutely fresh grazing for young stock each year and meticulous care over the disinfection of foaling boxes.

Differences in individual susceptibility to infection. The susceptibility of lambs to parasitic gastritis varies considerably in different individuals exposed to identical infection, but this does not appear to be the case with foals. A very similar degree of infection was developed by all foals on the same stud, the only exceptions being those which had suffered some setback early in life. Thus foal 18 was not getting enough milk from its dam and did not thrive from the start; in addition it suffered from an abscess which further retarded its progress. The egg count of this foal rose suddenly to 3,400 in 26 weeks, but it subsequently began to improve and the count went down later to about 1,000 e.p.g. In the same way, foal 23 was getting insufficient milk and at 16 to 17 weeks had a count of 2,500 e.p.g. Its diet was later supplemented with cow's milk and its egg count went down to less than 1,000 e.p.g. In this connexion it is of interest to note the apparent anthelmintic action of skim milk fed in large quantities to pigs (Spindler *et al.*, 1944). Ascarids, whipworms and nodular worms were expelled by pigs which consumed up to eight gallons of skim milk daily, but this may have been a purely mechanical sequel to the violent purging which accompanied the milk diet and there is no direct evidence of any true anthelmintic action. Apart from foals 18 and 23, exposure to infection appeared to be the only factor affecting the degree of infestation and there was no evidence that individual foals varied in susceptibility to the parasites.

Effect of weaning on course of infestation. A study of the detailed egg counts shows that in many cases there was a sudden rise in egg count 12 to 16 weeks after weaning as, for examples, in foals 1, 2, 22 and 26. This may be ascribed to an increased intake of parasitic larvae at weaning time when resistance is lowered by the shock, and when many foals will not feed for days. Three months later these worms are mature and start to lay eggs causing a marked rise in the egg count. These observations suggest that a resistant animal is able to prevent infective larvae from entering the mucous membrane and that any factors leading to lowered resistance will result in an increased infectivity of freshly acquired larvae which, once established, are no longer susceptible to the defence mechanism of the host.

Effect of anthelmintic treatment. All the foals on Studs A, D, E and F received some form of anthelmintic treatment at weaning

time, but a perusal of the weekly egg counts shows that this had little effect on the extent of the infection. The only treatment which appeared to be effective was that used at Stud D where the foals were given daily doses of 3 gms. phenothiazine in alternate weeks. In this case the drop in egg count probably resulted from the inhibitory action of the drug on egg laying as observed by Gibson (1945), and not from the actual expulsion of parasites. It is interesting to note that phenothiazine had little effect on the output of *Ascaris* eggs, already declining at this time.

It is common practice among thoroughbred breeders to dose their foals at weaning time, but from observations made in the course of this work little is gained by this treatment. For one thing, the foals are subjected to a further setback at a time when their resistance is already low and secondly, the majority of the parasites and especially the more harmful large strongyles have not reached maturity and are inaccessible to the action of any known drug. It would seem much more practical to dose the foals at about a year old just before putting them on to new pasture in the spring.

SUMMARY

Weekly examinations of the faeces of 26 thoroughbred foals and their dams from seven different studs provided the following information.

Eggs of strongyloid worms that appear in foals' faeces soon after birth are referable to coprophagia, which is practised to a considerable extent by all foals.

Evidence of genuine infection with redworms was first noted at 12 to 14 weeks and the eggs of the various species appeared in the following order: *Trichonema* spp. (12 to 17 weeks), *Poteriostomum* spp. (15 to 32 weeks), *Gyaloccephalus capitatus* (17 to 50 weeks), *Triodontophorus* spp. (18 to 46 weeks), *Strongylus vulgaris* (25 to 36 weeks), *Strongylus edentatus* (40 to 55 weeks). A key for the differentiation of the infective larvae of these species is included.

Trichostrongylus axei was found to occur on six studs where cattle were kept, but was absent from the 7th where only horses had been grazed for a number of years.)

Infection with *Strongyloides westeri* develops at an early age, even when foals are kept indoors with mares that are apparently free of the parasite. The infection in foals rises to a peak at six to ten weeks and falls rapidly from ten to 14 weeks, finally disappearing altogether at 15 to 23 weeks. The very low pathogenicity of this parasite is suggested by observations made on an experimental foal which at 22 days old had an egg count of 50,000 *Strongyloides* e.g., but continued in excellent condition. This foal rapidly acquired an immunity and was resistant to super-infection. An attempt to infect the dam also failed.

Ascaris equorum occurred in every foal, eggs being first passed

reached at about 16 to 18 weeks, after which the infection diminished from 19 to 28 weeks. In some cases all worms had apparently been expelled at six months, but in others infection persisted up to 52 weeks or more. The possibility of prenatal infection with this parasite is discussed.

There was no evidence of any relationship between the extent or type of infection in a foal and that in its dam.

The rate at which infestation was acquired was very similar in foals born in January to that in foals born in April and May.

The type of stud management and the extent to which pasture hygiene was practised were found to be deciding factors affecting the ultimate level of infection with redworms, but had no effect on the incidence of *Ascaris* infestation.

No evidence was obtained to indicate any marked difference in the susceptibility of individual foals to parasitic infestation, such as is known to occur in lambs.

Temporary lowering of resistance due to weaning or other setbacks, was observed to coincide with an increased development of infective larvae which was reflected by a rise in egg count three to four months later.

On the whole anthelmintic treatment was of no value and the common practice of dosing foals at weaning time is to be deprecated as it does not appear to achieve the desired result. The most dangerous species have not at that time reached maturity and are not accessible to the action of drugs in the intestine.

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THE OCCURENCE OF TUBERCULOSIS IN THE WILD WOOD-PIGEON

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INTRODUCTION

ALTHOUGH tuberculosis is of common occurrence in domestic and semi-domestic fowl, it has seldom been described in free-living wild birds in this country; cases which occurred, have been recorded by Feldman (1938), Griffith (1939) and Hignett and McKenzie (1940). Because of the numerous avian and non-specific tuberculin reactions which occur in cattle and swine, an enquiry regarding the possible existence of infection in wild birds due to acid-fast organisms was commenced at this Field Station in 1943; 360 birds of various species have so far been examined and tuberculosis has been found in the wood-pigeon (*Columba palumbus*, L), the rook (*Corvus frugilegus*, L) and the starling (*Sturnus*, *V. vulgaris*, L.). This paper records the cultural and pathogenic properties of strains of *Mycobacterium tuberculosis* isolated from wood-pigeons and describes the type of disease met with in that species.

MATERIAL AND METHODS

Sixty-six wood-pigeons were examined; 52 were shot in the vicinity of Compton, seven were obtained from the Edward Grey Institute of Field Ornithology, Oxford and seven from the Hon. Miriam Rothschild. The birds from the last two sources were selected for examination because of some external abnormality such as plumage change or emaciation.

At autopsy any macroscopic lesions were noted, films, sections and cultures prepared and the material examined biologically.

Microscopic Examination. Smears were stained by the Ziehl-Neelsen method, sections by the same technique and also with haematoxylin and eosin.

Cultural Examination. Abnormal tissue was emulsified in Griffith's tubes and a 1:10 suspension prepared in normal saline. A portion of this suspension was removed for biological test and the remainder treated with oxalic acid according to the method of Corper and Uyei (1929). Four slopes of 5 per cent. glycerol egg and plain egg were inoculated with the final precipitate and incubated at 37° C. Sub-cultures from the primary growths were made on coagulated ox serum, 5 per cent. glycerol ox serum, plain egg, 5 per cent. glycerol egg, 5 per cent. glycerol potato and also on a modified Petroff's medium. The media were examined for growth at weekly intervals.

Biological Examination. Guinea-pigs, rabbits, fowls and domestic pigeons were employed, and two of each species were inoculated with a

1:10 suspension of tissue in normal saline, except the rabbits which received a suspension of a three-week culture on ox serum medium standardised to an opacity matching Brown's tube No. 6. The guinea-pigs were inoculated intramuscularly, the fowls, pigeons and rabbits by the intravenous route and in all cases the inoculum was 0.5 ml.

Each animal, except the rabbits, was injected intradermally with 0.1 ml. of avian and mammalian P.P.D. tuberculin three weeks after inoculation. At *post-mortem* examination, cultures were made on glycerol egg and plain egg medium.

RESULTS

The macroscopic findings are shown in Table I and the biological tests in Table II.

Two birds shot at Compton (3.85 per cent.) four from Oxford and four sent by the Hon. Miriam Rothschild showed lesions resembling tuberculosis. Plumage change was noted in eight specimens, *i.e.*, the mantle appeared darker than normal and the feathers lacked their normal gloss. In most cases emaciation was marked, the infection being of a chronic type with yellow-orange tubercles, varying in size in different organs. Apart from the colour, these foci resembled those encountered in tuberculosis of the domestic fowl. Clusters of nodules similar to the grape-like lesions sometimes found on the serous membranes of cattle affected with bovine tuberculosis, were present on the visceral and parietal peritoneum of pigeon O. 1745. In this bird, particles of infected tissue had become detached from the peritoneum and small orange-coloured bodies were found free in the abdominal cavity. Lesions involving the intestinal tract were found in five of the ten cases; one bird, MR5, showed no visible macroscopic evidence of disease apart from emaciation and plumage change.

Smears and/or sections from each case showed the presence of acid-fast organisms morphologically similar to *M. tuberculosis avium* and the histological changes resembled those found in tuberculosis. Viable organisms were not recovered culturally or biologically from the two birds shot at Compton and O. 1675 and O. 1679 were unsuitable for culture or experimental inoculation. Thus, six strains were obtained, *viz.*, O. 1720, O. 1765, MR1, MR4, MR5, and MR7, which, with one exception (MR1), appeared to be typical avian strains. On primary isolation, growth was enhanced by the presence of glycerol and subsequently cultures similar to avian strains were obtained in about three weeks on all the media employed. Acid-fast organisms were not readily isolated from MR1 even in the presence of glycerol and coagulated ox serum eventually proved to be the most suitable medium for this strain.

As shown in Table II, all six pathogenic strains were fully virulent for fowls and rabbits, less so for pigeons and apparently incapable of producing progressive infection in the guinea-pig. Fowls, one pigeon and all the guinea-pigs gave a marked reaction to avian P.P.D. three weeks after infection and in some cases a slight

TABLE I
Post-mortem LESIONS

No. of Wood- pigeon	Source	Macroscopic Examination		Microscopic Examination		
		Plumage Change	Condition	Organs	Smears (stained Z.N.)	Section (stained H. & E.) (⁹ Z.N.)
C13	Compton	None	Slight atrophy of pectoral muscles	Caseous nodules in liver, pancreas, ventriculus, intestines and kidneys	Liver and intestinal nodule +	Ventriculus } TB. + Liver }
C53	Compton	None	Normal	3 large caseous nodules in spleen	Spleen +	Spleen } TB. +
O.1675	Oxford	D-NB	E	Small, flat nodules in liver. Enlarged spleen, 3 x 1.5 cm. with purulent foci and thickened fibrous tissue. Flat plaques beneath pericardium	...	Liver } TB. + Spleen }
O.1679	Oxford	D-NB	E	Multiple nodules in liver substance. Yellowish exudate on visceral surface of liver	...	Liver } TB. +
O.1720	Oxford	D-NB	E	Miliary lesions in liver. Nodules in intestines, pericardium, epicardium and pectoral muscles	Liver + Heart + Pectoral muscles —	Liver } TB. + Heart }
O.1745	Oxford	D-NB	E	Grape-like nodules on peritoneum and mesentery. Small nodules at ileo-caecal orifice and in liver, gizzard and spleen	Liver +	Liver } TB. + Spleen }
MR1	The Hon. Miriam Rothschild	D-NB	E	Fibrous, fat-like growths in liver, gizzard, pancreas, intestine, mesentery and pericardium	Peritoneal lesions +	Liver } TB. + Heart } Gizzard }
MR4	"	D-NB	E	Small caseous nodules in the spleen	Spleen +	Spleen } TB. +
MR5	"	D-NB	E	No macroscopic lesions	Liver +	Liver } TB. +
MR7	"	D-NB	E	Caseous nodules in gizzard, intestine, liver, spleen and kidneys	Liver +	Liver } TB. +

Key: + signifies masses of acid-fast bacilli.

E signifies emaciation.

... signifies not done.

TB. signifies typical tuberculous lesions (including endotheioid and giant cells).

D-NB signifies dark colour—no "bloom."

TABLE II

BIOLOGICAL TESTS

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Experimental Animals												
No. of Pigeon	Tissues examined	Fowl		Pigeon		Guinea-pig		Rabbit		Result	Result	Result
		TB. Test at 3 Weeks		TB. Test at 3 Weeks		TB. Test at 3 Weeks		TB. Test at 3 Weeks				
		A.	M.	A.	M.	A.	M.	A.	M.			
C13	"Pooled" sample	—	—	—	—	—	—	—	—	—	—	—
C53	Spleen	—	—	—	—	—	—	—	—	—	—	—
O.1720	"Pooled" sample	++	+	—	—	—	—	—	—	—	—	—
O.1745	"Pooled" sample	++	+	—	—	—	—	—	—	—	—	—
MR1	"Pooled" sample	++	+	—	—	—	—	—	—	—	—	—
MR4	Spleen	++	+	—	—	—	—	—	—	—	—	—
MR5	Liver	++	+	—	—	—	—	—	—	—	—	—
MR7	"Pooled" sample	++	+	—	—	—	—	—	—	—	—	—

Key: A signifies avian P.P.D. tuberculin.
M " " mammalian P.P.D. tuberculin.
— " " no reaction.
+ " " degree of reaction.
... " " not done.

N.TB. signifies no evidence of tuberculosis macroscopically, microscopically or culturally.
K " " killed.
D " " died.
TB. " " infection confirmed by smears and cultures.

reaction to mammalian tuberculin was also noted. The organisms were recovered in culture from the rabbits, fowls and the one pigeon which became infected, but no cultures could be obtained from the iliac and lumbar lymphatic glands of guinea-pigs killed six weeks after the original inoculation.

DISCUSSION

Tuberculosis of free-living wild birds in this country is apparently of rare occurrence and those cases which have been reported have seldom been confirmed culturally and/or biologically. Feldman (1938) in a comprehensive survey of avian tuberculosis mentions cases in various wild species but no instance of infection in the wood-pigeon is recorded. At Weybridge, Doyle (unpublished work) made observations on 47 wood-pigeons and found two infected. In 1943 the Hon. Miriam Rothschild informed the Imperial Bureau of Animal Health that she considered tuberculosis to be common in wild wood-pigeons and that infected birds could be distinguished from normal birds by their changed plumage. Colonel Hammerton, of the London Zoological Society, informed the Bureau that he examined seven wood-pigeons forwarded to him by the Hon. Miriam Rothschild; organ smears and the distribution of the lesions suggested that these were cases of tuberculosis.

In the present work, infection due to acid-fast organisms was detected in 10 of 66 wild pigeons. Despite efforts to recover organisms from the two birds obtained at Compton, no cultures were obtained and therefore it appears feasible either that the organisms in those two cases required special cultural methods and were non-pathogenic for laboratory animals or that they were no longer viable. Thus the possibility of the existence of another infection due to acid-fast organisms cannot be entirely excluded. McEwen (1939) and subsequently Taylor (1945) described a disease of sheep apparently caused by an acid-fast organism which was extremely difficult to cultivate on artificial medium. The well known "skin tuberculosis" in cattle is a similar condition in which, although acid-fast organisms can be observed in smears from the affected tissues, cultures are not obtained and experimental animals cannot be infected. Leprosy is yet another classical example of an acid-fast infection from which the causal organism cannot be cultivated (Muir, 1930). More recently, in Denmark, Christiansen, Ottosen and Plum (1946) encountered a condition in wild pigeons similar to tuberculosis from which cultures could not be obtained despite the presence of large numbers of acid-fast organisms in smears prepared from the lesions.

In the present work, with the exception of the two birds, shot at Compton, all the pigeons showed some alteration of plumage, the feathers, especially of the mantle, being darker than normal with a matt finish. This may be associated directly with the disease or indirectly as a result of lack of preening. This plumage change, although not infallible, is helpful in selecting possible cases of tuber-

culosis in wood-pigeons. Because of the number of birds showing involvement of the intestinal tract, it is reasonable to assume that wild wood-pigeons may contaminate pasture and water supplies, especially in downland areas where water is scarce and where wild pigeons congregate in large numbers at any available supply during the summer months. The non-specific reactions in a proportion of cattle subjected to the tuberculin test may be associated with the ingestion of these organisms.

The true extent of acid-fast infection in wild pigeons has not, so far, been accurately determined because of the difficulty of obtaining an adequate supply of material, but examination of the small number of unselected pigeons obtained at Compton (52) indicates an infection rate of 3.85 per cent.

SUMMARY

Ten cases of infection due to acid-fast organisms have been described in wild wood-pigeons (*Columba palumbus*, L.).

Plumage change was present in eight of the ten cases.

Macroscopic lesions were observed in the viscera of nine of the ten birds. The tenth case, apart from change of plumage and emaciation, showed no macroscopic abnormality at *post-mortem* examination.

Lesions were present in the intestinal tract of five of the ten pigeons.

Six strains of *Mycobacterium tuberculosis avium* were isolated from these birds. Two pigeons failed to yield cultures although large numbers of acid-fast organisms were observed in smears from the lesions.

In 52 wood-pigeons (unselected samples) the incidence of acid-fast infection was 3.85 per cent.

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THE USE OF PLASMA, INCORPORATED IN SOLID MEDIUM, FOR THE DETECTION OF COAGULASE POSITIVE STAPHYLOCOCCI OF BOVINE ORIGIN

By

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INTRODUCTION

THE power of staphylococci to coagulate plasma, as shown by Chapman, Berens, Peter and Curcio (1934) and numerous other workers, has been accepted as a reliable index of pathogenicity.

The usual methods of determining coagulase production by staphylococci are by means of the tube test, described in detail by Cruikshank (1937), and the slide technique of Cadness-Graves, Williams, Harper and Miles (1943) in which human plasma is used. More recently, Field and Smith (1945) have compared the value of various animal plasmas for these tests. A close correlation has been shown to exist between these two methods. The disadvantages are that sterile plasma is required to be kept on hand, diluted as needed and separate colonies have to be picked from primary plates.

Human plasma has been incorporated in solid medium by Penfold (1944) and Reid and Jackson (1945) and coagulase positive strains of staphylococci recognised by a halo of opacity at the periphery of the colonies. By this method pathogenic staphylococci can be recognised in primary cultures.

It was considered that this technique might be of value in the recognition of pathogenic staphylococci in primary cultures of milk and this paper records the efficiency of the solid medium method compared with the tube test for the purpose of distinguishing between coagulase positive and coagulase negative staphylococci of bovine origin.

METHODS

Origin of Strains. A total of 96 strains of staphylococci were obtained from (a) milk of cows suffering from clinical mastitis, not necessarily caused by staphylococci, (b) milk from apparently normal udders, (c) swabs from the teats of normal udders. In Table II these are shown respectively as "mastitis," "normal," and "teat swabs."

These strains, after primary isolation, were dried *in vacuo* over phosphorous pentoxide and when required for testing, were reconstituted in Hartley's broth and streaked on blood agar medium.

The characters of these strains after drying are shown in Table I.

The 96 strains were tested for chromogenesis by growing upon milk agar, (Christie and Keogh, 1940), and for haemolysis, by the method of Bryce and Rountree (1936). To test pathogenicity, five adult mice, either sex, approximately 22 g. in weight, were inoculated intravenously with 0.5 ml. of a 24-hour glucose broth culture. If

TABLE I
THE CHARACTERS OF THE STRAINS EMPLOYED

Total No. of Strains	Colour	Type of Haemolysis	Pathogenicity in Mice
91	Orange	$\alpha\beta$: 71 Nil : 20	59 0
4	White	$\alpha\beta$: 2 Nil : 2	1 0
1	Yellow	Nil : 1	0

three or more mice died within a week, the strain was considered to be pathogenic.

Tube Test: As described by Topley and Wilson (1936).

Solid Medium: A 3 per cent. agar base was used with 25 per cent. rabbit, sheep or ox plasma and 0.1 per cent. of a 1 per cent. aqueous solution of neutral red. The latter tinted the medium slightly and showed up the opacity. Penfold (1944) advocates pouring the medium as thinly as possible, but this was not found to be an advantage for shallow plates became dry and cracked. In contrast, plates of 9 c.m. diameter, containing 12 ml. medium gave satisfactory readings even after storage in the icebox for one month, whereas Cruikshank (1937) recommends that plasma for the tube test be used within 14 days. The surface of the plate was dried before inoculation. Areas approximately 4 mm. in diameter, were heavily inoculated and up to six strains were tested on one plate. Readings were taken after 18 hours incubation at 37° C.

RESULTS

Table I indicates the principal characters of 96 strains of staphylococci. Of the 73 strains shown to be haemolytic, 60 were pathogenic for mice. There were 23 non-haemolytic, non-pathogenic strains. Thus a total of 60 pathogenic and 36 non-pathogenic strains were available for assessing the comparative values of the tube and solid medium tests.

TABLE II
PATHOGENICITY, COAGULASE TUBE TEST AND SOLID MEDIUM TEST

Origin of Strain	Total No. of Strains	Pathogenic Strains	Positive Coagulase Tube Test			Positive Solid Medium			Non-pathogenic Strains	Coagulase Tube and Solid Medium Tests		
			R	S	O	R	S	O		R	S	O
Mastitis	35	33	32	32	31	32	31	27	2	All tests negative		
Normal	33	18	18	18	18	18	18	9	15			
Teat swabs	28	9	9	9	6	9	8	6	19			
Total	96	60	59	59	55	59	52	42	36			

R = Rabbit plasma. S = Sheep plasma. O = Ox plasma.

Table II shows the results of both coagulase methods using rabbit, sheep and ox plasma. It will be seen that the solid medium is as reliable as the coagulase tube test when rabbit plasma is employed. Sheep plasma is less reliable and ox plasma gives variable and delayed results. This confirms the findings of Field and Smith (1945) using the tube and slide tests. The standard by which these tests are judged is that of the actual pathogenicity of the organism found by mouse inoculation. Thus, to judge the comparative efficiency of these tests the percentage association is calculated by comparing each test with the pathogenicity. These comparisons are shown in Table III which shows that 98 per cent. of the total pathogenic strains were detected both by the tube and solid medium tests using rabbit plasma. None of the 36 non-pathogenic strains were shown to be virulent by either tube or solid medium tests (Table II). In the case of sheep and ox plasmas the results were less reliable and consequently these methods were discarded.

TABLE III

<i>Origin of Strain</i>	<i>Positive Coagulase Tube Test</i>			<i>Positive Solid Medium Test</i>		
	R	S	O	R	S	O
Mastitis ...	97	97	94	97	94	82
Normal ...	100	100	100	100	72	50
Teat swabs ...	100	100	67	100	89	67
Totals ...	98	98	91	98	87	70

Thus the correlation between the two methods using rabbit plasma was absolute, both for the detection of pathogenic and non-pathogenic strains.

Direct Plating of Milk: In addition to the 96 strains tested as described, a number of samples of milk were tested in primary culture. Of the fresh sample of milk, 0.5 ml. was inoculated on to a solid medium rabbit plasma plate which was then dried. After 18 hours incubation at 37° C. it was found that coagulase-positive staphylococci could be detected by the halo of opacity with which the colonies were surrounded.

SUMMARY

Ninety-six strains of staphylococci, 60 pathogenic and 36 non-pathogenic, from bovine milk and teats were examined for coagulase production.

A solid medium incorporating rabbit, sheep and ox plasma respectively was used to detect coagulase-positive staphylococci and a comparison made between this method, the tube test and pathogenicity for the mouse.

It was shown that the use of rabbit plasma in solid medium was effective in detecting coagulase-positive staphylococci of bovine origin.

It is suggested that for direct plating of milk this method might be used in the detection of potentially pathogenic staphylococci of the cow's udder.

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THE FATE OF A SULPHONAMIDE, SODIUM SULPHACETAMIDE, IN THE INCUBATING EGG

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INTRODUCTION

MANY organisms associated with diseases of both man and animals are of low virulence or non-virulent for the more commonly used laboratory animals. A number of these organisms can, however, be established in, and are often pathogenic to, the incubating egg: they include *Streptococcus agalactiae* which is associated with the more prevalent form of bovine mastitis. The possibility of assessing the therapeutic value of sulphonamide drugs against this organism using the developing egg for *in vivo* tests was considered. As the value of these drugs depends on the presence in the tissues for an adequate time of a concentration sufficient to exert a therapeutic effect, it was thought desirable to investigate the fate and toxicity of these substances in the incubating egg before proceeding to protection experiments.

In this study the sulphonamide chosen was sodium sulphacetamide; sulphanilamide was rejected on account of its low solubility.

Although many workers have evaluated prophylactic and therapeutic agents in experimental infections of the chick embryo, the fate of sulphonamides in the incubating egg has not been studied so fully. Morrow and Bang (1938) and Bang and Bang (1941) investigated sulphanilamide, sulphathiazole and sulphapyridine therapy of gonococcal infections of the chorio-allantoic membrane. Colorimetric tests revealed the presence of the drugs throughout the egg with only slight destruction, but analyses at 24 and 48 hours accounted for only half the dose administered. Weil and Gall (1941) chose the sodium salt of sulphathiazole in treating *Shigella* infection of the embryo. Eggs were dosed by injection of the drug into the yolk or amniotic sac. By the former route 10 to 70 per cent. of the drug was absorbed within six hours and 40 to 90 per cent. by the latter route and the L.D. 50 was in the region of 40 mg. per cent. Francis *et al.* (1947) studied the relative potencies of the more common sulphonamides against *Str. agalactiae* in the chick embryo and found there were no large differences in the blood levels produced by the various drugs.

METHODS

Eggs of 10 to 11 days incubation with vigorous embryos were used. They were examined for viability at 12 hourly intervals and unless mentioned in the text, were prepared for analysis 48 hours after incubation.

Using the technique described by Burnet (1933), dilutions of the drug in 0.1 or 0.2 ml. quantities were pipetted on to the chorio-allantoic membrane through a window cut in the egg shell. Another route of inoculation, namely injection of the drug directly into the allantoic sac, was also investigated.

The free sulphacetamide, which is the active compound, was estimated by the method described by Bratton and Marshall (1939). In order to facilitate transport and to ensure thorough mixing of tissues, the incubating eggs, excluding the shell, were dehydrated

TABLE I
TOXICITY

<i>Dose</i>	<i>Alive</i>	<i>Dead</i>	<i>Total</i>	<i>Per cent dead</i>	<i>Batch No.</i>
5 mg. per cent.	2	0	5	0	1
	3	0			2
7.5 mg. per cent.	3	0	3	0	2
10 mg. per cent.	2	0	5	0	1
	3	0			2
15 mg. per cent.	2	1	13	30.71	2
	1	2			3
	3	1			6
	3	0			9
20 mg. per cent.	0	2	25	40.0	1
	2	1			2
	2	4			3
	6	1			8
	5	2			10
25 mg. per cent.	3	5	30	36.67	3
	3	2			5
	5	1			7
	5	2			8
	3	1			9
30 mg. per cent.	2	4	27	40.79	3
	5	1			6
	3	3			7
	4	2			8
	2	1			9
35 mg. per cent.	4	3	16	43.75	7
	5	4			10
40 mg. per cent.	5	3	13	53.8	4
	3	2			9
45 mg. per cent.	3	5	12	66.7	4
	1	3			6
50 mg. per cent.	1	4	5	80.0	5
60 mg. per cent.	0	5	5	100.0	6
75 mg. per cent.	0	5	5	100.0	5

by grinding with a weighed amount of anhydrous sodium sulphate in the proportion of 2 g. of salt to 1 g. of egg material. Sodium sulphate did not interfere with the assay. Weighed aliquots of this mixture were diluted 20 times with distilled water, the proteins precipitated with 15 per cent. trichloroacetic acid (4 ml. to gramme of the egg/sulphate mass) and the mixture filtered. The colorimetric assay was then carried out on the filtrate, solutions of sodium sulphacetamide of known strength being used as standards. Control incubating eggs, subjected to the same treatment with the exception that physiological saline replaced the drug solution, were included in each experimental batch.

To estimate the free compound in the distribution studies, the various embryonic fluids were removed from their respective sacs with Pasteur pipettes and collected in sterile tubes, a few drops of chloroform being added as a preservative. The embryo was then removed, washed free of amniotic fluid and placed, with a few drops of chloroform, in a small container.

The sulphacetamide content of the embryo and embryonic fluid was determined by the method of Bratton and Marshall (1939). It was found impossible to estimate the total sulphacetamide as the colour produced after acid hydrolysis was of a different shade from that given by the free sulphacetamide thus rendering colorimetric measurement inaccurate.

RESULTS

Toxicity of the drug. The lethal dose (L.D. 50) of sodium sulphacetamide administered by the chorio-allantoic route was determined by giving a series of doses to groups of eggs selected at random. The results obtained are shown in Table I and by plotting the percentage mortality against dose, the L.D. 50 was approximately 35 mg. per cent.

Distribution and fate of the drug. By estimating the amount of free drug present 48 hours after administration of various doses it was found that approximately 75 per cent. of the drug had been inactivated and the extent of this inactivation was similar in both dying and surviving eggs.

TABLE II
RECOVERY OF FREE SODIUM SULPHACETAMIDE 48 HOURS AFTER
CHORIO-ALLANTOIC ADMINISTRATION

Egg No.	Dose	Recovery of free drug	Per cent dose unchanged	State of embryo
2	15.0 mg. per 100 gm.	4.50 mg. per 100 gm.	30.0	Alive
7	20.0 " "	6.43 " "	32.15	"
16	25.0 " "	5.17 " "	20.7	"
23	30.0 " "	7.14 " "	23.8	"
4	15.0 " "	3.75 " "	25.0	Dead
9	20.0 " "	4.73 " "	23.65	"
18	25.0 " "	6.43 " "	25.7	"
24	30.0 " "	10.20 " "	34.0	"

By direct injection of the drug into the allantoic sac much larger doses were tolerated without harming the embryo and the proportion of drug remaining unchanged was much higher.

TABLE III
RECOVERY OF FREE SODIUM SULPHACETAMIDE 48 HOURS AFTER
INTRA-ALLANTOIC INJECTION

<i>Egg No.</i>	<i>Dose</i>	<i>Recovery of free drug</i>	<i>Per cent. dose unchanged</i>	<i>State of embryo</i>
6	10 mg. per 100 gm.	4.50 mg. per 100 gm.	45.0	Alive
10	20 " "	8.30 " "	41.5	"
18	30 " "	14.10 " "	47.0	"
23	40 " "	19.5 " "	48.75	"
27	50 " "	24.13 " "	48.1	"

The difference in these results was explained when the distribution of the drug in the embryo and in the fluids of the embryonic system was studied. It was found that a greater concentration of drug reached the embryo and the amniotic fluid by absorption from the chorio-allantoic membrane than from intra-allantoic injection. In the latter case most of the drug remained in the allantoic fluid, thus accounting for the lower toxicity of the drug when given by this route.

TABLE IV
DISTRIBUTION OF SODIUM SULPHACETAMIDE 48 HOURS AFTER ADMINISTRATION
BY DIFFERENT ROUTES

<i>Route</i>	<i>Dose</i>	<i>Concentration of free drug</i>		
		<i>Embryo</i>	<i>Amniotic fluid</i>	<i>Allantoic fluid</i>
Chorio-allantoic	Mg. per cent.	Mg. per 100 gm.	Mg. per 100 gm.	Mg. per 100 gm.
	45	38.4	18.0	27.0
	45	22.2	47.0	21.0
	40	56.4	79.0	33.0
	40	16.8	13.0	82.2
Intra-allantoic	20	2.60	6.14	30.0
	30	7.80	24.0	121.5
	40	4.5	13.7	50.8
	50	3.6	28.0	18.0

Rate of Absorption and Distribution. Of the two methods of administration, the chorio-allantoic route gave a higher drug concentration in the embryo and would seem to be the desirable method for chemotherapeutic tests. To give an indication of the rate of absorption, distribution and inactivation of the drug, incubating eggs dosed with 5 mg. were taken every six hours and prepared for analysis. One egg was prepared for total analysis by dehydrating with anhydrous sodium sulphate and the embryo and its fluids collected from another.

TABLE V

RECOVERY OF FREE SODIUM SULPHACETAMIDE IN THE WHOLE EGG AT 6-HOURLY PERIODS FOLLOWING ADMINISTRATION OF 5 MG.

Period, hours	Egg No.	Net weight, gms.	Recovery of free drug
6	102	49.91	6.75 mg. per 100 gm.
12	104	52.13	2.83 " "
18	106	47.28	4.72 " "
24	108	49.61	2.73 " "
30	110	53.54	4.20 " "
36	112	51.515	3.6 " "
42	114	47.50	3.15 " "
48	116	52.49	3.24 " "
54	118	49.53	4.35 " "
60	120	48.74	3.15 " "
66	122	49.32	3.36 " "
72	124	49.89	2.25 " "

Although there were wide variations in the results, free sodium sulphacetamide was still present in the embryonic system 72 hours following administration. The greater part of the inactivating process was shown to take place within 12 hours after which time the amount of drug in the egg seemed to remain fairly constant.

TABLE VI

ABSORPTION AND DISTRIBUTION OF SODIUM SULPHACETAMIDE FOLLOWING CHORIO-ALLANTOIC ADMINISTRATION

Egg No.	Time	Concentration of free drug			
		Embryo	Amniotic fluid	Allantoic fluid	Yolk
	Hours	Mg. per 100 gm.	Mg. per 100 gm.	Mg. per 100 gm.	Mg. per 100 gm.
101	6	7.50	1.35	2.64	1.3
103	12	2.61	1.80	13.04	1.9
105	18	4.50	2.5	4.32	1.6
107	24	2.85	2.58	4.56	1.5
109	30	2.40	2.4	4.05	1.36
111	36	3.60	4.05	37.50	2.16
113	42	3.00	2.04	22.50	2.40
115	48	1.12	1.86	23.70	2.50
117	54	3.63	1.12	2.00	0.22
119	60	1.12	0.07	1.12	0.32
121	66	1.10	2.91	5.52	1.92
123	72	1.09	1.00	3.00	1.5

Pathological Changes observed in the Experimental Eggs

As soon as possible after death, the egg was opened and an examination made of the embryo and associated structures. Where death was not anticipated examination was carried out 48 hours after the administration of the drug. Tissues and organs for section were removed and fixed in Helly's Fluid. In the case of the embryo, it was found best to fix it whole after removing the limbs and opening the abdominal wall when serial sections could be cut to include all the organs. All sections were stained with haematoxylin and eosin.

Macroscopic Lesions. Where death resulted from the large doses macroscopic lesions were marked. The chorio-allantoic membrane

was thickened, the blood vessels were slightly engorged and in the very high doses there was extravasation of blood. The blood was very dark in colour but was re-oxygenated quickly on exposure to air.

The embryo showed marked changes in the higher doses. The subcutaneous blood vessels were very evident and the blood was dark. The liver was mottled with haemorrhagic areas and in some cases had pale necrotic margins. The heart wall was opaque and haemorrhages into the pericardium were sometimes seen. The most striking and constant lesion, however, was the congestion of the kidneys. Even in the lower doses this was often noticed and in the higher doses was most marked, the kidneys being enlarged and haemorrhagic.

Microscopic Changes. In those doses which did not cause death there were no obvious histological changes although the kidney sometimes showed a small degree of congestion. In doses from 20 mg. per cent. upwards, lesions varied in severity proportionate to the size of the dose.

Dose, 20 mg. per cent. The sinusoids of the liver were filled with blood but the parenchyma was not distorted and no degenerative changes were seen in the liver cells. The kidney showed some interstitial infiltration although the cells of the glomeruli and tubules appeared normal. There was a certain amount of debris in the lumen of the tubules.

Dose, 40 mg. per cent. The liver showed marked changes. Vacuolation of the cells had occurred and areas of necrosis, especially near the margins of the organ, were evident. The blood channels were much enlarged and packed with blood. The kidneys were engorged with blood and the glomerular tufts were in various stages of degeneration. There was a marked interstitial infiltration of white cells. The spleen was congested.

Dose, 75 mg. per cent. With this dose it was extremely hard to recognise the structure of the various organs. The individual blocks of liver cells were widely separated and necrotic. The cells of the heart muscle were swollen and degenerated and the kidney was completely broken up, glomeruli and tubules being in an advanced state of degeneration. These changes closely resemble those recorded by Fisher and Haag (1942) following sulphacetamide dosing of mice.

DISCUSSION

From these experiments it appeared that inoculation of sodium sulphacetamide on to the exposed chorio-allantoic membrane was the most convenient and desirable method of administration. Absorption from the allantoic sac was irregular, and thus resembled similar findings recorded by Weil and Gall (1941) after injection of sodium sulphathiazole into the yolk sac. Using the chorio-allantoic route it was possible to attain a reasonably uniform concentration of sodium sulphacetamide in the embryo, which persisted for at least 48 hours. The distribution of the compound in the embryonic system was irregular, the concentration of the drug in the embryo

and embryonic fluids bearing no constant relationship to one another. When assessing the value of therapeutic agents such as the sulphonamides in experimental infections of the embryo, the concentration of the active substance in the embryo itself is of primary importance. Hence, in such experiments, the use of a therapeutic index, calculated as follows, is suggested. Therapeutic index:

Dose preventing death in 50 per cent. of infected embryos
L.D. 50 of the drug

where embryos had been infected with a lethal inoculum of the test organism.

More than 50 per cent. of the sulphacetamide was destroyed in the first 12 hours following inoculation, after which the concentration of free compounds, both in the whole egg and in the embryo, remained fairly constant for at least 48 hours. A similar destruction of sulphanilamide, sulphathiazole and sulphapyridine was recorded by Bang and Bang (1941). It might be thought that as such a large loss occurs, the incubating egg is not such a suitable test medium as the mouse, but this disadvantage is largely outweighed by the fact that many organisms associated with disease of man and animals, although non-virulent to mice, cause death of the chick embryo.

SUMMARY

Sodium sulphacetamide injected into the developing hen's egg, was absorbed more completely by the embryonic system after administration on to the exposed chorio-allantoic membrane than by other routes.

Only about 25 per cent. of the dose remained in the free form 48 hours after inoculation.

Distribution in the various structures of the egg was irregular, but the concentration in the embryo, following an initial fall 12 hours after inoculation, was fairly constant for 40 hours.

The L.D. 50 of sodium sulphacetamide was 35 mg. per 100 g. and pathological changes produced in the egg resembled those found in mice.

The use of the incubating egg in chemotherapeutic experiments is discussed.

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BRUCELLA INFECTION AMONG EWES, CAMELS AND PIGS IN EGYPT

By

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INTRODUCTION

As further steps in the survey of brucella infection in the milch animals in Egypt (Zaki, 1948), the milks of ewes and she-camels were examined. In Egypt it is the custom to breed and rear sheep with goats so that in the mixed flocks of the peasantry the chances of the ewes contracting infection from close proximity with the goats, in which the evidence of brucella infection varies but occasionally becomes high, is great. A proper survey of the incidence of infection in goats throughout the country, however, awaits to be completed. She-camels in milk are the main, and in some circumstances the only source of milk to the bedouin Arabs, who lead a nomadic life wherever pasture and water can be found in the desert. Although sheep are kept mainly for breeding lambs for meat, their surplus milk is often mixed with buffalo milk for human consumption.

INCIDENCE OF BRUCELLA IN THE MILK OF SHEEP

Dubois (1931) pointed out that brucella infection of sheep was accompanied by abortion, but that the majority of ewes did not abort more than once, though some aborted twice and, exceptionally, more than two abortions occurred. Ananaidis and Miaoulis (1931) described an outbreak of epizootic abortion in ewes in Greece in which only newly imported ewes between the age of two and four years were attacked. Affected ewes gave a positive agglutination blood serum titre with *Br. abortus* at dilutions of 1 : 100 to 1 : 1,000. Schroeder (1930) reported sheep and goats to be susceptible to *Br. abortus* infection through contact with infected cattle. Most outbreaks of abortion among sheep, as is now well known, have been proved to be caused by infection with organisms other than brucella, such as vibrionic abortion. Topley and Wilson (1936) summarise the situation by stating that experimentally the disease can be produced in sheep, but that natural infection with *Br. abortus* seems to be rare.

EXPERIMENTAL

Evidence of brucella infection in Egyptian sheep was sought by examining their milk for the presence of organisms and their specific agglutinins, and by testing their blood serum for specific agglutinins.

One hundred samples of milk were collected from ewes, kept with goats and cattle (but not buffaloes) in several parts of Cairo

and district. Equal amounts were taken from each half of the gland, using precautions similar to those described in the preceding paper (*loc. cit.*) to exclude extraneous contamination. On arrival at the laboratory the samples were immediately examined by direct culture of centrifuged milk sediment and cream, by testing the whey for agglutinins and by guinea-pig inoculation.

Two hundred blood samples were collected from ewes slaughtered at Cairo abattoir. The serum was tested against suspensions of *Br. abortus* and *Br. melitensis* for agglutinins.

RESULTS

The results of the milk examinations were entirely negative in that brucella could not be cultivated from any of the samples; the agglutination test on the whey failed to demonstrate the presence of agglutinins at a titre above 1:5, and the organisms could not be recovered from any of the inoculated guinea-pigs (spleen or lymph gland). The serum of the inoculated guinea-pigs did not agglutinate suspensions of *Br. abortus* at a titre above 1:20.

The results of the blood examinations are summarised in the following table:—

TABLE I
AGGLUTINATION TESTS ON BLOOD SERA OF EWES

Highest serum titre	Number of ewes showing serum reactions
1:20	19 (9.5 per cent.)
1:40 and above	None

Assuming that the ewes were exposed to brucella infection from the goats and cattle living in very close contact with them, the presence of agglutinins in a small proportion (9.5 per cent.) of the slaughtered ewes at a titre not exceeding 1:20, would suggest that they possessed a high degree of resistance to such brucella infection as prevailed among the other species of animals. It may, of course, be that in the outbreaks recorded in the literature in which the abortions were traced to brucella infection, the strains of brucella responsible had become specially highly adapted to sheep. It is, in any event, noteworthy that in a ruminant community in which the incidence of brucella infection is high, sheep usually escape infection.

INCIDENCE OF BRUCELLA INFECTION IN CAMELS IN EGYPT

It is difficult in Egypt to obtain a sufficient number of milk samples from she-camels upon which to make an investigation, because all the camels used by Government departments are males.

Only ten samples could be collected for this work and not more than 20 c.c. could be obtained in any one case. The she-camels were nearing the end of their lactation. Blood was obtained at the same time. On arrival at the laboratory, a few drops of each milk sample were spread on each of two liver agar plates containing 1:200,000 gentian violet. The plates were incubated in a CO₂ atmosphere for four days at 37° C., after which they were examined: any brucella-like colony was subcultured and subjected to further examination bacteriologically. The rest of each sample was inoculated intraperitoneally into two male medium-sized guinea-pigs. After six weeks the guinea-pigs were killed and cultures made from their spleens and lymph glands on appropriate media.

Brucella could not be isolated from any of the samples by either method, although the blood serum of one animal gave a positive agglutination titre with *Br. abortus* rising to 1:1,600. The other nine sera were negative. The number of samples tested, however, was too small to draw any definite conclusions.

To obtain further evidence of the incidence of infection, tests were carried out on the blood serum of camels slaughtered at Cairo abattoir. Two hundred samples were collected for this purpose, 100 from males and 100 from females. The agglutination test was carried out in the usual way, using a suspension of *Br. abortus* containing 1,000 million organisms per c.c. The results are summarised in Table II.

TABLE II
AGGLUTINATION TEST ON BLOOD SERA OF CAMELS

Highest serum titre	Number of camels showing positive reactions	
	Males (total 100)	Females (total 100)
Negative or below 1:25	86 per cent.	74 per cent.
Positive 1:25	8 "	12 "
" 1:50	3 "	4 "
" 1:100	1 "	4 "
" 1:200	1 "	0 "
" 1:400	0 "	3 "
" 1:800	1 "	1 "
" 1:1,600	0 "	2 "

The proportion of camels showing positive serum reactions to brucella, together with the range of the titres recorded, suggests that camels are far more susceptible than sheep to brucella infection, even when they are not living in such intimate association with goats and cattle in which natural susceptibility to infection and incidence of infection are commonly high. Whether, however, the milk of camels is commonly infected with brucella is unknown.

A NOTE ON BRUCELLA INFECTION IN PIGS IN EGYPT
CAUSING CYSTIC OVARIES AND STERILITY

While the author was carrying out his enquiry into the incidence of brucella among the milch animals of Egypt, an outbreak of sterility and abortion was brought to his notice in 1942 among the pigs of a large breeding establishment in Upper Egypt. As a preliminary test, blood sera from five sows affected with sterility after repeated service were tested for brucella agglutinins, four giving a positive reaction at 1:200 dilution, and one at 1:400. These five sows were then slaughtered and blood samples taken for bacteriological and serological examination. At *post-mortem* examination, in one of the sows, the ovaries each measured 10×15 cm., and appeared to consist almost entirely of about ten large cysts containing a transparent,



FIG. 1. Cystic Ovaries of Sow. On the right, a series of ovaries from sows in the same outbreak showing graded degrees of encystation are displayed. All $\frac{1}{8}$ natural size.

blood-tinged fluid under tension. No change was observed in other parts of the reproductive tract or elsewhere in the body. The ovaries in the other four sows varied in size, up to about three to four times the normal and were also cystic (Fig. 1). Cultures made from the blood on ordinary and on liver agar were incubated aerobically and in CO_2 ; all failed to show any growth of brucella. Likewise, cultures made from a few drops of the cyst contents spread on to the same media and incubated in the same way failed to show growth. About 2 c.c. of the cyst fluid were also inoculated intraperitoneally into each of two medium-sized male guinea-pigs, which were killed after six weeks. Cultures were made from the spleen and lymph glands with negative results. Agglutination tests upon the cyst contents

using suspensions of *Br. abortus* and *Br. melitensis* proved negative. Nothing could be seen in the centrifuged deposits of the cyst contents except some red blood corpuscles and haemoglobin crystals.

A boar suffering from inflammation and enlargement of the testicles, was castrated; a strain of brucella was isolated from the epididymis aerobically on liver agar. On subculture, the strain continued to produce H_2S for four days and grew well on liver agar containing 1:50,000 thionin, but did not grow on agar containing 1:25,000 fuchsin. The strain would, therefore, conform to *Br. suis*, according to Huddleson's differential tests for classification.

Later, in 1942, 12 more sows from the above herd were slaughtered because of persistent sterility. Their blood sera agglutinated *Br. abortus* to titres ranging from 1:200 to 1:1,000, except for one case which was negative. The ovaries of all these sows were also cystic. Cultures made from the blood and cyst contents again proved negative in all cases.

Blood sera from 13 sterile sows were examined with the following results: agglutination at 1:800, 2; at 1:400, 1; at 1:200, 4; at 1:100, 1; at 1:50, 1; at 1:25, 2. Two failed to agglutinate at 1:25.

Sera from the eight boars used on the establishment were also tested: four proved free from brucella agglutinins, three agglutinated at 1:200, and one at 1:400.

Samples of blood taken from all the mature sows among the pigs on the farm (approximately 5,000) disclosed a high proportion of brucella infection in the herd.

An aborted foetus from a sow about three months advanced in pregnancy, was despatched in glycerine to the laboratory for examination. *Br. suis* was isolated from the stomach contents and heart blood.

Attempted vaccination.—An attempt was made to vaccinate some of the healthy young breeding sows in the herd.* A formolised vaccine (0.05 per cent.) was prepared from a 48-hour broth culture of *Br. abortus* and *Br. suis*, each sow receiving two successive injections intramuscularly of 10 and 20 c.c. respectively at a week's interval. The sows were not served until three months after vaccination. Of the 75 vaccinated animals, one aborted and 14 proved to be sterile and had to be sent to the abattoir, while the remaining 60 sows gave birth to normal litters. The ovaries of the 14 sterile sows were cystic and some were distinctly fibrous and atrophied to about one-third to one-fourth the normal size. No clear cut evidence was, therefore, forthcoming that vaccination reduced the incidence of disease; unfortunately, it was not possible to obtain comparative figures relating to the incidence of abortion in unvaccinated controls.

* Undertaken in conjunction with Dr. Zaki Morcos, who attended the herd clinically.

Effect of Br. suis upon female guinea-pigs.—Five healthy female adult guinea-pigs were each inoculated subcutaneously with 0.25 c.c. of a fresh 48-hour broth culture of the strain of *Br. suis* isolated from the above outbreak. Five more females were similarly inoculated with the same dose of the same culture after it had been killed in a water bath at 60°C for one hour. Two females of the same lot were reserved as uninoculated controls.

Six months later, all the animals were killed and the ovaries examined. No abnormalities could be detected in the reproductive tract or ovaries of the two uninoculated controls or of the controls inoculated with the dead culture. The ovaries of all five females inoculated with the live culture, however, showed discrete, pin-head sized, transparent vesicles dotted over the surface.

Histological sections were examined from all the ovaries. In the case of both the uninoculated controls and those inoculated with the dead culture, the Graafian follicles presented a normal appearance, showing ova in different stages of development; the lining cells of the ripe follicles were healthy and intact (Fig. 2). On the other hand, some of the ovaries from the guinea-pigs which had been inoculated with live culture, showed very distinct cystic changes in the Graafian follicles which were free from ova, the lining cells being detached from the basement membrane and undergoing degeneration. The inoculation of female guinea-pigs with *Br. suis* can, therefore, cause disturbance in the ovarian cycle, leading to cystic formation of the Graafian follicles (Fig. 3).

DISCUSSION

The possible relationship between brucellosis and sterility or infertility in the bovine female has been discussed at length in the literature. Nowhere, however, has the relationship been so clearly established as in the pig where, as shown above, brucellosis caused by *Br. suis* type of organism was responsible not only for overt abortion but also for a very high incidence of sterility, in which normal ovulation was prevented by cyst formation in the ovaries. The mechanism of the disturbance, however, was not clear. It could not be attributed to direct infection of the Graafian follicles with brucella. The experiments on guinea-pigs suggest that failure to rupture and discharge healthy ova appeared to arise through some agency which prevented normal oögenesis, and operating remotely, either elsewhere in the reproductive tract so as to cause an upset to the normal rhythm or in other tissues. It may well be that what is so apparent in the infected sow also transpires, but less obviously, in the cow. Against that view is the very large experience that has accumulated upon the effect of the inoculation of guinea-pigs with *Br. abortus*. We can trace no record pointing to the production of striking ovarian malformations; such guinea-pigs, however, are usually killed for examination after an interval not exceeding two

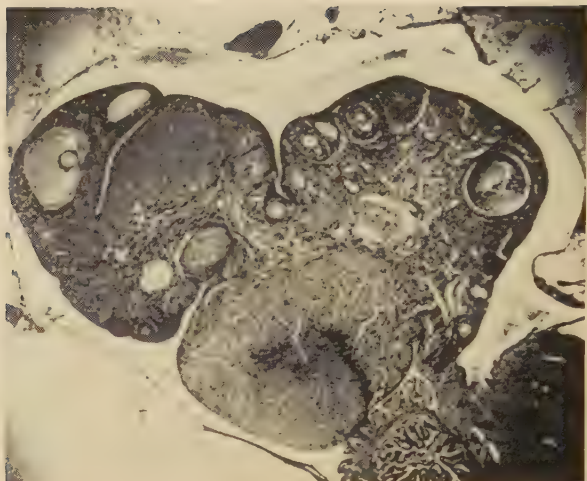


FIG. 2. Ovary of Control Guinea-Pig, 6 months after inoculation with dead *Brucella suis*, showing normal development of Graafian follicles. $\times 80$.

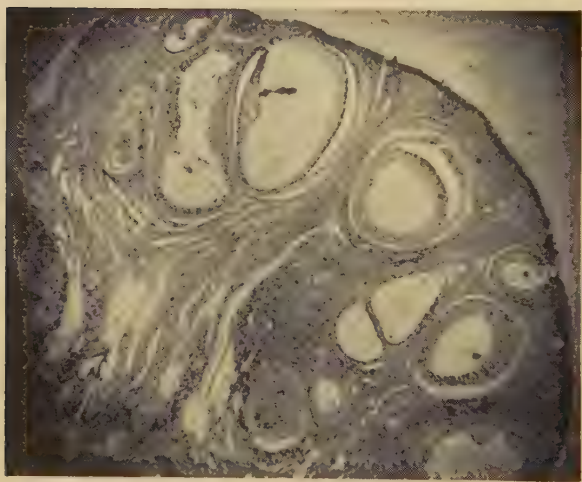


FIG. 3. Ovary of Experimental Guinea-Pig (much enlarged in comparison with normal ovary in Fig. 2), 6 months after inoculation with live *Brucella suis*, showing encystation in Graafian follicles. $\times 80$.

months after inoculation. The evidence would, therefore, suggest that *Br. suis* is more prone than *Br. abortus* to cause ovarian disturbances. It is striking that on this establishment a large number of buffaloes were also kept, but no history of abortion was obtained. Hence it would appear that the strain of *Br. suis* was of very low infectivity for bovines.

CONCLUSIONS

Brucella could not be isolated from the milks of 100 ewes kept in close proximity with goats and cattle in Cairo and district.

The blood sera of 200 ewes were subjected to the agglutination test: 90.5 per cent. were negative, while 9.5 per cent. did not exceed a titre of 1:20. It is possible that sheep possess a high degree of resistance against natural infection from the other species.

Ten samples of milk from she-camels examined by direct culture and guinea-pig inoculation proved negative. The blood serum titre of one of these camels was high (1:1,600), but the remainder were negative.

Blood serum taken from 200 camels (100 males and 100 females) showed the presence of specific agglutinins at titres ranging from 1:25 to 1:1,600 in 14 per cent. of the males and 26 per cent. of the females. Camels would, therefore, appear to be relatively highly susceptible to *Br. abortus* infection.

An outbreak of brucellosis among the pigs of a large breeding establishment in Upper Egypt, caused by a *Br. suis* type of organism, is described.

Infection caused abortion and a very high incidence of sterility which was traced to cyst formation in the ovaries. Some of the boars were also infected, with the production of lesions in the epididymis.

The strain of *Br. suis* produced cystic ovaries in guinea-pigs killed six months after inoculation.

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ALLOXAN DIABETES IN SHEEP

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INTRODUCTION

It has been observed by Shaw Dunn, and McLetchie (1943) that the intravenous administration of alloxan to the rabbit produced a specific necrosis of the beta cells of the islet tissue of the pancreas and disturbance of carbohydrate metabolism like that in diabetes mellitus. Alloxan diabetes has since been demonstrated in the rat (Dunn and McLetchie, 1943), dog, cat, guinea-pig, pigeon (Goldner and Gomeri, 1943) and sheep (Jarrett, 1946).

The animals of an experimental group show a variable response to the same dose level of alloxan, some being refractory to its action, some succumbing within a few hours and some passing into a stage of chronic diabetes (Lackey *et al.*, 1944; Duffy, 1945; Lazarow and Palay, 1946). It has been established that a single injection of the drug may produce a triphasic response in the level of blood glucose, an initial hyperglycaemia, a transient hypoglycaemia within 24 hours after administration, and a final hyperglycaemia which may persist until death (Goldner and Gomeri, 1943). The mechanism of this triphasic response has not been fully elucidated although there is fairly general agreement that the final stage is due to necrosis of the beta cells of the islet of Langerhans.

EXPERIMENTAL METHODS

Adult Blackface sheep kept under normal management were used. No attempts were made to starve the animals before treatment in order to make them uniformly susceptible (Kass and Waisbren, 1945), since it is impossible to clear the alimentary canal of ruminants even by prolonged starvation.

Alloxan (B.D.H.) was prepared as a 5 per cent. solution immediately before the injection of doses from 20 mg./kg. to 200 mg./kg. into the recurrent tarsal vein. Blood for examination was drawn from the jugular vein, and after immediate precipitation of the proteins by Van Slyke's modification of Folin-Wu's tungstic acid method, glucose was estimated by the Shaffer-Hartmann modification of Somogyi's copper-iodometric method. Glucose and ketones in the urine were estimated by Benedict's and Rothera's tests respectively.

All organs except the pancreas were fixed in formal saline. Thin (5 mm.) slices of the pancreas were fixed in Bouin for 12 hours before transference to 70 per cent. alcohol. All sections except those of the pancreas, were stained with haematoxylin and eosin and a modified Picro-Mallory method. The pancreas was stained by many methods, including those described by Gomeri (1941).

The intravenous glucose-tolerance test for cattle elaborated by Bell and Jones (1945) was shown to be applicable to sheep, the technique used being the same. A constant dose of 0.2 gm./kg. glucose was injected intravenously at a uniform rate of 20 ml. per minute using a 50 per cent. solution of anhydrous glucose (Bell and Jones, 1945).

RESULTS

The toxic dose of alloxan for sheep

Varying amounts of alloxan were inoculated intravenously with the results shown in Table I. It will be seen that alloxan given intravenously to sheep in doses greater than 80 mg./kg. live weight is fatal. A large dose of 200 mg./kg. causes death within 24 hours. The animals were hypoglycaemic and the symptoms presented were lethargy, prostration, convulsions (jerking of the neck and paddling of the limbs) and coma. One of the animals (No. 7) showed violent but intermittent convulsive symptoms for two hours before death. Small doses of alloxan (25 mg./kg.) had no apparent toxic effect, and in one instance a dose of alloxan of 50 mg./kg. produced no ill effects. Those sheep that became diabetic and eventually succumbed, rapidly lost as much as 50 per cent. of the pre-experimental weight. Normal appetite was maintained but marked thirst developed. Polyuria was noticeable and the urine contained glucose and ketones. The blood glucose levels of sheep Nos. 14 and 20 are illustrated in Fig. 1, and are typical of the diabetic level produced by the intravenous administration of alloxan in a dose of 100 mg./kg.

TABLE I
TOXIC EFFECTS OF ALLOXAN ON SHEEP

<i>Sheep</i>	<i>Alloxan, mg./kg.</i>	<i>Survival period in days</i>	<i>Blood glucose level at death</i>
7	200	15 hours	Hypoglycaemic
23	200	15 hours	"
24	200	18 hours	"
1	150	7 days	Hyperglycaemic
17	125	17 days	"
28	100	6 days	"
21	100	8 days	"
14	100	10 days	"
20	100	10 days	"
9	100	11 days	"
24	100	13 days	"
2	100	14 days	"
6	100	25 days	"
5	80	90 days	"
30	50	113 days	"
29	50	Survived	Normal
25	25	"	"
26	25	"	"

The blood sugar response

The effect of varying doses of alloxan on the blood sugar concentration during the first seven hours after injection is shown in Table II. It will be seen that a dose of 80 mg./kg. live weight or more produces hyperglycaemia within two hours. In one case,

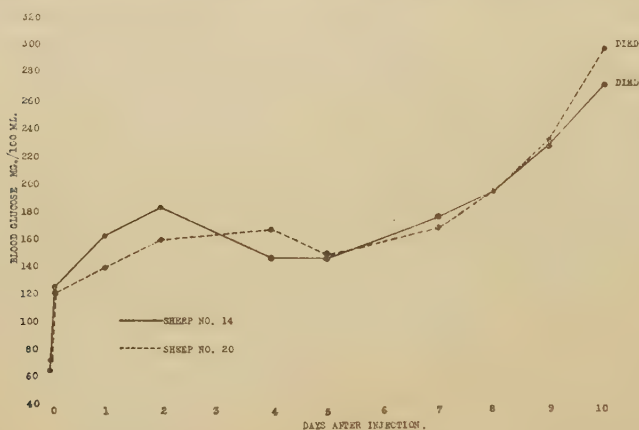


FIG. 1.—Blood glucose values in sheep following the intravenous injection of 100 mg./kg. of alloxan.

No. 30, 50 mg./kg. produced a similar effect. Only in animals receiving the high doses of alloxan did the initial hyperglycaemia fall to normal or to a state of hypoglycaemia. Sheep receiving 200 mg./kg. showed, after the initial hyperglycaemic phase, a very depressed blood glucose level (less than 8 mg./100 ml.), which

TABLE II
BLOOD SUGAR RESPONSE FOLLOWING THE INTRAVENOUS INJECTION OF
ALLOXAN TO SHEEP

Sheep	Dose alloxan in mg./kg. body weight	Blood glucose, mg./100 ml.									
		Pre-injection	Hours after injection								
			1	2	3	4	5	6	7	8	
7	200	55	99	101	119	90	94	46	46	14*	
1	150	50	62	96	136	101	92	88	58	71	
21	100	69	94	156	189	238	—	280	278	334	
6	100	50	69	88	140	151	—	182	180	221	
28	100	62	78	96	180	216	—	214	171	218	
24	100	58	112	180	223	247	243	241	238	267	
5	80	58	85	94	101	110	119	112	119	94	
29	50	42	42	62	55	60	50	65	48	58	
30	50	39	88	114	121	125	116	94	94	101	
25	25	57	57	57	48	48	38	—	45	56	
26	25	58	50	48	16	44	53	48	62	53	

* Blood glucose value when animal was moribund 15 hours after alloxan administration.

persisted until death. This premortal hypoglycaemia was confirmed in all sheep which died following the administration of 200 mg./kg. alloxan. The blood glucose level of animals which received a diabetogenic dose of alloxan was raised to values above 120 mg./100 ml. and remained at a high level until they died. The hyperglycaemia is illustrated in Fig. 1, but even higher values were found in other sheep; No. 9, for example, had a blood glucose value of over 450 mg. per 100 ml. for several days before it died.

Glucose tolerance tests

It will be seen from Table III and Fig. 2 that sheep given the diabetogenic dose of 100 mg./kg. alloxan intravenously did not lose, to any great extent, their ability to clear the injected glucose from the blood. Although in the tests the pre-injection blood sugar levels were high, after alloxan most of the excess blood glucose was cleared in two hours. A part of the glucose was voided in the urine, since the blood glucose levels were much above the renal threshold. The removal rate of glucose from the blood, as calculated by the method described by Hamilton and Stein (1942), indicated there was no striking impairment in clearance of glucose from the blood after alloxan treatment.

TABLE III
GLUCOSE CLEARANCE TESTS ON SHEEP GIVEN 100 MG./KG.
ALLOXAN INTRAVENOUSLY

Sheep number	Time of test after administration of alloxan	Blood glucose mg./100 ml.						Removal rate of glucose % per minute
		Pre-injection	Time after injection, minutes					
			2	15	30	60	120	
2	Before injection	73	165	127	125	101	94	2.9
	2 hours	169	310	258	243	218	189	3.1
	5 days	216	302	276	267	247	236	3.1
	11 days	149	214	214	221	—	206	2.0
9	Before injection	65	162	129	125	94	88	3.8
	2 hours	158	285	280	247	249	214	2.9
	5 days	232	308	—	282	278	278	2.4
20	Before injection	78	145	119	110	101	88	3.1
	2 hours	125	173	180	173	173	162	2.4
	1 day	173	236	210	206	210	191	2.4
	2 days	160	223	193	189	165	187	2.3
14	Before injection	67	136	112	85	73	62	3.9
	2 hours	127	200	184	180	182	173	2.3
	1 day	173	236	210	206	210	191	2.4
	2 days	184	280	254	252	243	169	4.6

Post-mortem EXAMINATION

Morbid anatomy

In those sheep which died in the acute stage of alloxan toxæmia, *i.e.*, in hypoglycaemic coma, no abnormalities were observed. The

animals which became diabetic showed marked loss of flesh and extreme emaciation in those cases in which hyperglycaemia persisted for a prolonged period. There was an excess of fluid in the pericardial, pleural and peritoneal cavities. The liver and kidneys were of normal shape and size but were buff coloured. Cataract, which occurs in human diabetes and in alloxan diabetes of rabbits (Duffy, 1945), was not observed.

Histo-pathology

No microscopic lesions were seen in those sheep which died in the acute stage of alloxan toxæmia.

Liver.—In sheep which became diabetic, the parenchymal cells showed extreme and coarse vacuolation, indicating fatty degeneration. In No. 5, necrosis of the individual liver cells was marked and they contained large acidophil droplets. Glycogen could not be demonstrated histo-chemically.

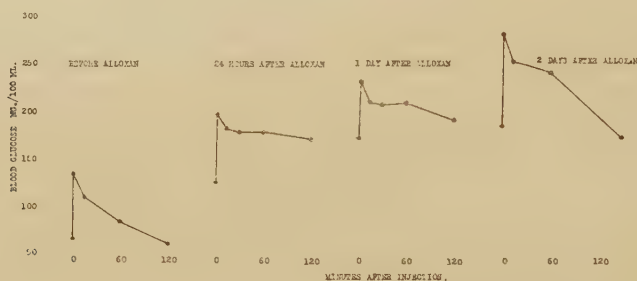


FIG. 2.—Intravenous glucose-tolerance curves of Sheep No. 14 following the intravenous injection of 100 mg./kg. alloxan. In all cases 0.2 gm./kg. glucose was injected at a uniform rate of 20 ml. per minute. The upright arrow indicates the end of the injection period.

Kidney.—Owing to the extremely rapid onset of *post-mortem* autolysis due to the heat insulation afforded by the fleece, it was difficult to interpret fully the histological findings. In the diabetic sheep, Bowman's capsule contained relatively large masses of protein and the capsular basement membrane was thickened. The epithelium of the proximal convoluted tubules showed varying changes from swelling with vacuolation to extreme necrosis and stripping of the tubular epithelium. The epithelium of Henle's loop and the second convoluted tubules also showed all stages of cellular necrosis. Intratubular casts were common.

Spleen.—The pulp was congested and relatively increased in amount. The germinal centres were active but not hyperplastic. Oedema was a feature of the changes in this organ.

Lymph glands.—Large eosinophilic cells with swollen cytoplasm of irregular outline giving a syncytial appearance, were present in the germinal centres,

Pancreas.—The islets of Langerhans appeared to vary in number and size in sheep. It was not possible to stain selectively either the alpha or beta cells. In No. 5 normal numbers of islets within the normal range were still present, but most of the nuclei were pycnotic.

Pituitary.—The gland appeared normal except for a relative increase in the number of the basophil cells of the *pars anterior*. No basophil cells were seen in sheep No. 5.

Adrenal.—This organ was apparently normal in all sheep except No. 5, in which the cells of the medulla were shrunken with darkly staining nuclei and with acidophil droplets present both within and outside the cells.

Thyroid.—This organ appeared to be normal in all cases except No. 5, in which the epithelial cells showed vacuolation of cytoplasm with pycnosis of their nuclei; in many cases the epithelium was stripped and the dead cells lay within the vesicle among fragments of stringy colloid. Where the epithelium was relatively healthy there was some evidence of attempts at proliferation.

DISCUSSION

The results of the experiments described above show that alloxan is diabetogenic for sheep, as it is for non-ruminant animals, thus confirming the findings of Jarrett (1946). The minimum dose of alloxan, injected intravenously, required to produce the diabetogenic effect was 50 mg./kg. live weight. Sheep made diabetic by alloxan eventually died after a varying period of sustained hyperglycaemia, and no evidence of spontaneous recovery from the diabetic condition such as occurs in the rabbit (Duffy, 1945) was observed. In the experiments described the diabetic sheep showed hyperglycaemia, glycosuria, ketonuria and polyuria and a gradually developing inanition, as is the case in rabbits made diabetic by alloxan (Duffy, 1945). Jarrett (1946), on the other hand, found that sheep made diabetic by alloxan did not show any marked signs of an acute deterioration in condition. Lenticular cataract was not observed.

Sheep show the triphasic blood glucose response which is characteristic in non-ruminant animals following alloxan administration. The triphasic blood sugar response, however, is not invariably produced by an effective dose of alloxan in sheep. The second phase, that of hypoglycaemia, which follows the initial hyperglycaemia usually within a few hours after the administration of the drug, may not occur. Relatively large doses of alloxan, 150 mg./kg. live weight and more, produce the secondary hypoglycaemia and when the dose is 200 mg./kg., the animals are unable to survive the severe hypoglycaemic coma which is produced. When relatively small doses of 25 mg./kg. are administered, there is no response other than a slight depression of the blood glucose level such as would be produced in normal sheep by insulin (Hitchcock and Philipson, 1947).

In sheep which died in hypoglycaemic coma induced by relatively large amounts of alloxan, no lesions were observed on *post-mortem*

examination or on histological examination of the organs. In the diabetic sheep it was not possible to demonstrate any specific lesion of the beta cells of the islet tissue as was shown to occur in other species (Shaw Dunn *et al.*, 1943), but both liver and kidney showed marked degenerative changes.

The induction of hypoglycaemic coma in sheep with definite symptoms of nervous derangement such as convulsions and subsequent death, is noteworthy, for Hitchcock and Philipson (1947) were unable to produce insulin shock in sheep showing hypoglycaemia for some hours following prolonged insulin therapy. Jarrett (1946), who demonstrated hypoglycaemia without symptoms of distress in alloxanised sheep, suggests that the pancreas of the sheep is unable to produce sufficient insulin to depress the blood glucose level and so to maintain it at a sufficiently low level to allow the ultimate symptoms to intervene. The experiments described above in which large amounts of alloxan were used, appear to show that a fatal hyperinsulinism can be produced.

Intravenous glucose clearance tests on sheep rendered diabetic by alloxan show no diminished tolerance for glucose in the early stages of the condition. Duffy (1945) showed that in alloxan-treated rabbits there was a diminished tolerance of glucose at various stages of the condition.

The experiments recorded here would appear to show that when alloxan is administered intravenously to ruminants a dysfunction of carbohydrate metabolism is produced which is similar to the effects of the drug on non-ruminant animals. Although it is now recognised that the carbohydrate available to ruminants for their body metabolism is not entirely derived from carbohydrate fractions of their food, it is probable that the intermediary carbohydrate metabolism is similar to that of non-ruminant animals.

SUMMARY

Alloxan is diabetogenic for sheep when amounts equivalent to 50 mg./kg. live weight are injected intravenously.

Sheep made diabetic by alloxan show symptoms similar to those of diabetes mellitus.

There is no spontaneous recovery in sheep from the diabetogenic action of alloxan.

A dose of 200 mg./kg. of alloxan causes death in sheep after a period of hypoglycaemia, which produces nervous symptoms and coma.

The results suggest that the intermediate carbohydrate metabolism of sheep is similar to that of non-ruminants.

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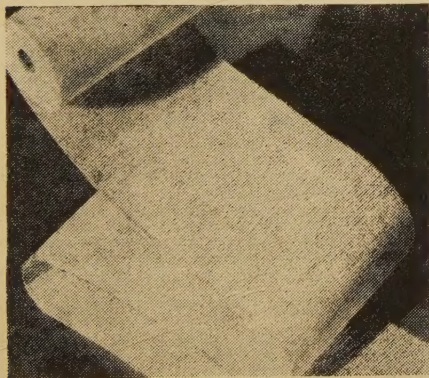
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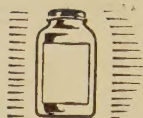
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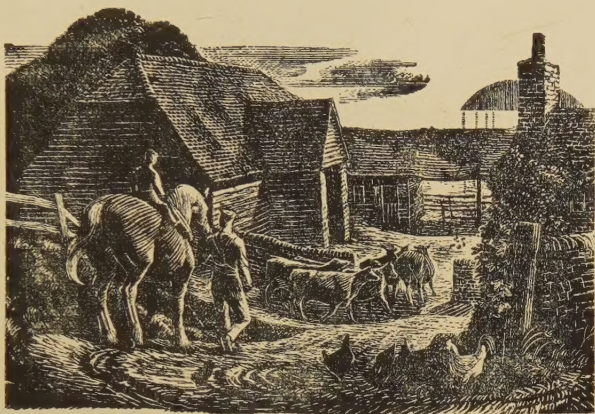
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